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SECONDARY SPORES IN *CORTICIUM EFFUSCATUM*[1]

BY MILDRED K. NOBLES[2]

Abstract

Corticium effuscatum Cooke & Ellis is heterothallic and tetrapolar. Oedocephaloid conidiophores bearing uninucleate conidia are developed on both haploid and diploid mycelia. Conidia produced on a haploid mycelium germinate only rarely to give new mycelia, but they are able to diploidise a suitable haploid mycelium when in association with it. A small percentage of the conidia produced on a diploid mycelium germinate to give haploid mycelia, part of which are identical in pairing reactions with one parent, the remainder with the other parent. Chlamydospores are produced on haploid and diploid mycelia and reproduce the generation on which they are borne.

Introduction

The production of conidia by *Corticium effuscatum* Cooke and Ellis in culture was admirably described and illustrated by Lyman (10). This work, however, predated the modern knowledge of sexuality in the Hymenomycetes, first stated by Bensaude (1) and Kniep (9), and lacks certain information regarding interfertility phenomena and distribution of nuclei, without which it is impossible to obtain an adequate understanding of the life history of the fungus, and in particular of the function of the conidia. The present study was undertaken to determine these additional facts.

Cultural Studies

Haploid Mycelium

Cultures were obtained from two fruit bodies, collected by Dr. R. F. Cain, the first (University of Toronto Herbarium No. *6503*) on *Alnus incana* at Holland River Marsh, Ont., October 3, 1934, the second (U. of T. Herbarium No. *6502*) on wood of a deciduous tree near New Durham, Ont., October 29, 1934. Both sporophores yielded heavy deposits of globose basidiospores, 5.3–7.0 μ in diameter. These, contrary to Lyman's experience, germinated readily in distilled water, 2% malt extract solution, and on agar containing 2% malt extract, producing a vesicle which either elongated directly or branched immediately, leaving the spore at the side of the germ tube. After the sporeling had achieved a considerable size, with several branches, simple septa appeared; the formation of this type of septum has continued in mycelia

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Contribution from the Department of Botany, University of Toronto, Toronto, Ont. Part of a thesis submitted to the School of Graduate Studies, University of Toronto, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Junior Plant Pathologist, Division of Botany and Plant Pathology, Central Experimental Farm, Ottawa, Canada.*

arising from single basidiospores during the more than seven years that these cultures have been maintained.

On medium containing 2% malt extract and 2% agar the mycelium from a single basidiospore grows slowly, producing a white mat, at first scanty and consisting of scattered fibres, later more plentiful, the newer growth raised, cottony, tufted, the older mycelium collapsed and woolly, or farinaceous because of the masses of conidia.

Conidia

The formation of conidia begins within two or three days after the germination of the basidiospores or transfer of the mycelium to fresh agar, and continues throughout the life of the mycelium. As in *Fomes annosus* (Fries) Cooke (*Trametes radiciperda*), the conidiophores of which were first described by Brefeld (4) and subsequently by many other authors, and in *Peniophora Allescheri* Bres. studied by the author (12), the conidia are borne on oedocephaloid conidiophores (Fig. 1) which are abundant on all aerial hyphae, occurring singly or in coremium-like aggregations. The heads are globose, 6.5–10.0 μ in diameter, the distal portion being covered with the slender, tapering sterigmata, each bearing a conidium. The conidia are usually crowded on the conidiophore, their number ranging from 2 to 30 per head, but averaging about 20. Lyman (10, p. 178 and Plate 21, Figs. 87 to 91) has described and illustrated the successive stages in the development of a conidiophore and its spores, and has noted the proliferation and branching of conidiophores, which is frequently observed. Following the formation of conidia, the conidiophores and cells from which they arise appear vacuolate and finally empty. The conidia are hyaline, thin-walled, globose like the basidiospores but smaller in size, 2.3–3.8 μ in diameter, averaging 2.75 μ. No spore deposit is produced from mycelia bearing conidia, so it must be assumed that the conidia, unlike the basidiospores, are not projected forcibly from the sterigmata.

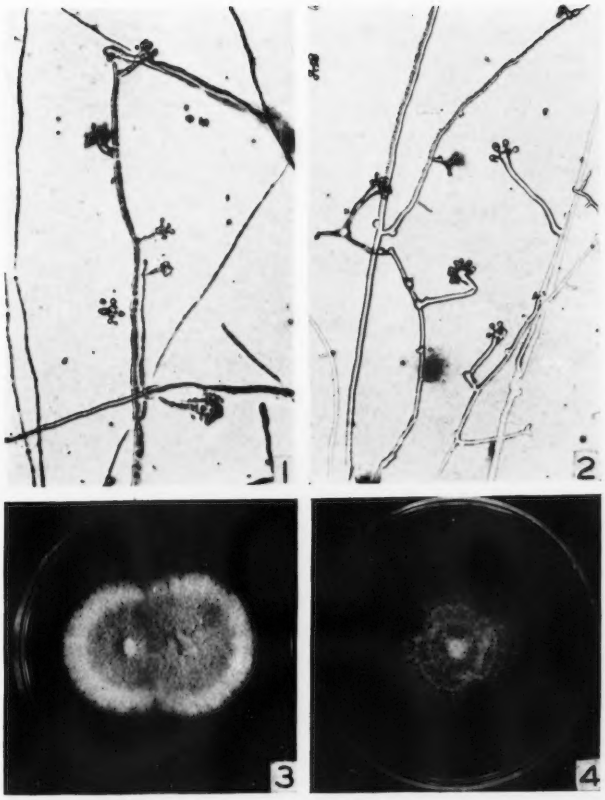
Chlamydospores

Numerous chlamydospores occur on the aerial and submerged hyphae of all monosporous mycelia. These are produced in an intercalary or terminal position by the rounding up of a portion of a cell, the concentration of the cytoplasm in that enlarged part, and its enclosure within a wall. The chlamydospores are broadly ovoid to lemon-shaped, with walls slightly thickened, 7.5–18.0 $\times$ 7.5–16.5 μ.

Pairings of Monosporous Mycelia

Five weeks after their isolation, 24 mycelia, each derived from the germination of a single basidiospore from fruit body No. *6503*, were grown together in pairs in all possible combinations and the resulting mycelia examined for the presence of clamp connections. The results are shown in Fig. 5, in which a plus sign indicates the occurrence of clamp connections, a minus sign their absence. It is evident that the mycelia fall into four groups, the members of one group reacting with the members of only one other group in such a way

PLATE I



FIGS. 1 TO 4. *Corticium effuscatum.* FIG. 1. *Haploid hyphae and conidiophores.* ×*400.* FIG. 2. *Diploid hyphae and conidiophores.* ×*400.* FIG. 3. *Pairing of two compatible haploid mycelia 10 days after inoculation.* ×*0.5 approximately.* FIG. 4. *Haploid colony diploidised by means of suitable conidia.* ×*0.5 approximately.*

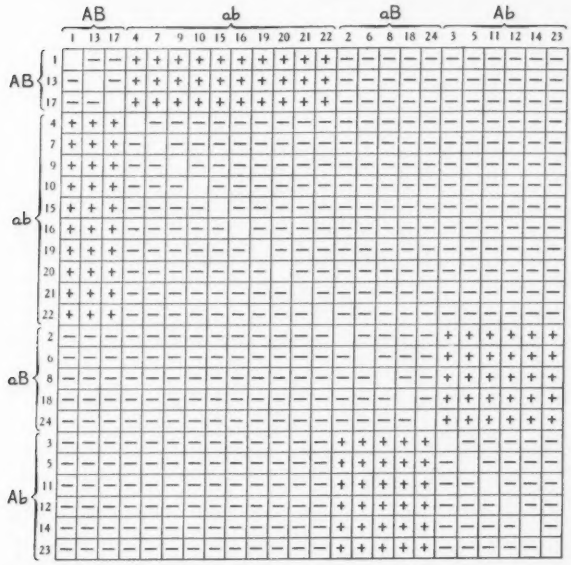


FIG. 5. *Corticium effuscatum. Results obtained by pairing in all possible ways 24 mycelia, each derived from the germination of a single basidiospore.*

as to form mycelium bearing clamp connections. Hence *Corticium effuscatum* is heterothallic and of the tetrapolar type of interfertility reaction. Where tetrapolarity occurs it has been assumed that the interfertility reactions are determined by two pairs of factors which are designated by the symbols *Aa* and *Bb*. The mycelium originating from the germination of a single basidiospore will then contain nuclei of one of the following constitutions *AB*, *ab*, *Ab*, *aB*. Two mycelia, which when grown together produce a mycelium with clamp connections, must contain nuclei of complementary constitution, *AB* + *ab* or *Ab* + *aB*. Thus in Fig. 5 the formula *AB* has been assigned arbitrarily to Mycelia 1, 13, and 17, from which it follows that Mycelia 4, 7, 9, 10, 15, 16, 19, 20, 21, and 22 must be designated by the formula *ab* and the other two groups by *aB* and *Ab* as shown. These results were corroborated by a second series of pairings of 16 cultures, each derived from a single basidiospore of fruit body No. *6502*. Here again the mycelia could be divided into four groups.

Four haploid mycelia of collection No. *6503*, one of each reaction group, were paired with four corresponding mycelia from collection No. *6502*, and the resulting mycelia examined for the presence of clamp connections. The findings are incorporated in Fig. 6, from which it is seen that clamp connections were formed in every pairing. This indicates that in *Corticium effuscatum*, as in many other species in the Hymenomycetes, which have been studied in a

similar manner, complete interfertility obtains when monosporous mycelia derived from fruit bodies from different localities are grown together.

		6503			
		1	7	2	3
6502	3	+	+	+	+
	1	+	+	+	+
	2	+	+	+	+
	4	+	+	+	+

FIG. 6. *Corticium effuscatum. Results obtained by pairing four haploid mycelia from fruit body No. 6502 with four haploid mycelia from fruit body No. 6503.*

Diploid Mycelium

Diploidisation of compatible haploid mycelia takes place fairly rapidly. Fig. 3 is a photograph taken 10 days after pairing of Mycelia 1 and 9 from collection No. *6503* (Fig. 5). It may be observed that there is a complete intermingling of the two mycelia at the line of meeting, and that the more abundant and fluffy diploid mycelium composed of hyphae bearing clamp connections, has formed an evident border around the colonies.

When subcultured on malt extract agar, the diploid mycelium forms a slightly more vigorous colony than the haploid, as shown by the formation of more plentiful aerial mycelium and the more rapid increase in the diameter of the colony. Microscopically the diploid mycelium resembles the haploid in production of chlamydospores and conidia but differs by having a clamp connection at every septum. The conidiophores and conidia are indistinguishable from those borne on haploid mycelium (Fig. 2).

When about five or six weeks old, such diploid colonies form fruiting surfaces similar to fructifications produced in nature. No detailed study of their development was made, but it was observed that basidia and gloeocystidia are produced in abundance and quantities of basidiospores are formed which are shot from the sterigmata in the usual way, giving heavy spore deposits. It would appear, however, that the mechanism for spore discharge frequently fails to function in this species, with the result that many basidiospores remain on the surface and become embedded by the subsequent proliferation of the hymenial layer. These embedded spores, an important diagnostic character of *Corticium effuscatum*, were termed "chlamydospores" by Burt (7), although Lyman (10) had described them earlier as "discharged basidiospores which are always imbedded in the growing hymenium". That they are basidiospores is borne out by the observation of the close agreement in size and shape between the embedded spores and basidiospores attached to basidia and in spore deposits, and by the fact that in young fruiting layers in culture, sufficiently loosely arranged to allow for careful microscopic examination of the component parts, the embedded spores are never attached to hyphae, in the manner in which chlamydospores occur, nor are they of the size and shape of the true chlamydospores of this species. The "antler-like" hyphae (dichophyses) usually present in fruit bodies of *C. effuscatum*, and which are the

basis for the transfer of this species to the genus *Asterostromella*[1] by Bourdot and Galzin (3) were not noted in cultures, although they may have escaped notice since no prolonged search for them was made.

Cytological Studies

A cytological examination has been made of basidiospores, sporelings, haploid and diploid mycelium, chlamydospore formation and stages in the development of conidiophores and conidia, using the technique previously described by the author (12).

Basidiospores

Basidiospores scraped from a spore deposit and mounted directly in Bouin's solution were found, on staining with Haidenhain's haematoxylin, to be binucleate (Fig. 9). Although it has not been demonstrated cytologically it seems clear from the experimental results set forth above that the two nuclei are of the same genetic constitution, and are presumably sister nuclei, derived from a mitotic division of the single nucleus that entered the spore during its formation.

Haploid Mycelium

On germination the two nuclei of the spore migrate into the vesicle (Fig. 10), which elongates (Fig. 11). Nuclear divisions follow to give the four-nucleate (Fig. 12) and finally, many-nucleate stages. Septum formation is delayed so that the young sporeling contains several nuclei (Fig. 13) with relatively few septa and these appear to be formed without reference to the nuclei. This multinucleate condition persists throughout the life of the haploid mycelium. Terminal cells in actively growing cultures have been observed to contain up to nine nuclei, while cells at a distance from the tip have been seen with one, two, or more nuclei.

Chlamydospores

Chlamydospores are produced in abundance in haploid hyphae, and in stained preparations are very prominent since their dense cytoplasm retains the stain after it has been removed from both the cytoplasm and nuclei in vegetative hyphae. During their formation, a small amount of vacuolate cytoplasm remains in the cells (Figs. 14 to 16) but when development is complete the cells appear empty. From one to eight nuclei have been observed in chlamydospores and since no divisions have been seen, it seems probable that they contain only the nuclei that were present in the cell in which they originated. This is substantiated by the observation that the chlamydospores containing many nuclei (Fig. 16) are always formed from multinucleate terminal cells.

[1] *As pointed out by G. W. Martin (Iowa Acad. Sci. Trans. 44: 48. 1938) the genus Asterostromella v. Höhn. & Litsch. is antedated by Vararia Karst. of which Corticium investiens (Schw.) Bres. is the type species. If the presence of these dichophyses, together with the other characters, can be shown to indicate relationship with other species, C. effuscatum should perhaps be included in Vararia. Since no combination is yet available, it has seemed best for the purposes of this paper to retain the species in Corticium.*

Conidia

A developing conidiophore can be distinguished from a branch by its broad base and more deeply staining cytoplasm (Fig. 17). The initial number of nuclei in a conidiophore formed on haploid mycelium is the same as that in the cell from which it developed. These migrate to the tip of the conidiophore and there divide (Figs. 18 to 19). Conidiophores have been seen in which nuclear divisions had been in progress when fixation occurred but figures of these have not been included because of the very small size of the nuclei. Following each division the nuclei appear successively smaller and after the 8- or 12-nucleate stage it is no longer possible to ascertain the exact number and position of nuclei within the head. However the nucleus can be seen more clearly after its migration into the spore, and it is apparent that the conidia are uninucleate.

Diploid Mycelium

The cells of the hyphae bearing clamp connections are consistently binucleate (Fig. 20), the nuclei dividing through the agency of the clamp connections. Chlamydospore formation proceeds as in the haploid mycelium, the chlamydospore always containing the two nuclei of the dikaryon (Figs. 21, 22).

The stages in the development of conidiophores on the diploid mycelium are similar to those in haploid mycelium. The young conidiophore, recognizable by its enlarged base and deeply staining contents, receives the two nuclei of the parent cell (Fig. 23). These divide (Fig. 24) without the intervention of a clamp connection, to give a four-nucleate structure (Fig. 25), the nuclei of which divide successively to give eight (Fig. 26) and 16 or more nuclei. As in the haploid conidiophore it is not possible to count the nuclei definitely beyond the eight-nucleate stage, but after the conidia are mature it is possible to distinguish *one nucleus* in each conidium (Fig. 27). It seems evident from these cytological observations, taken together with the experimental results reported below, that the binucleate clamp-bearing mycelium produces conidiophores in which the nuclei of the dikaryon, without preliminary fusion, divide successively to provide numerous nuclei, one of which migrates into each conidium. As in *Peniophora Allescheri*, studied by the author (12), the binucleate mycelium gives rise to uninucleate conidia.

Germination of Chlamydospores and Conidia

Chlamydospores

No special study has been made of germinating chlamydospores beyond the observation that they germinate very readily and vigorously. Twenty-four hours after being sown in hanging drop cultures of malt extract solution they had produced sporelings of considerable size, with several cells. Chlamydospores produced on a haploid mycelium germinate to give mycelia identical with it; those produced on a diploid mycelium give rise to hyphae bearing clamp connections immediately.

Conidia

First attempts to procure germination of conidia from a haploid mycelium in hanging drop cultures of malt extract solution met with complete failure. Observations were continued for nine days, during which the conidia remained unchanged. Lyman (10) encountered similar difficulties but observed (p. 181) that "the presence of growing hyphae appears to exercise a stimulating influence on the conidia". This suggested that they might act as diploidising agents, a function that Brodie (5, 6) has described for the oidia of *Coprinus lagopus* and *Collybia velutipes*. To test this the following experiment was carried out.

A haploid mycelium was allowed to grow on malt extract agar in a Petri dish for four days, the colony then being approximately 2.5 cm. in diameter. At that stage a drop of a suspension of conidia from a compatible haploid mycelium was placed at each of three marked spots on the periphery of the colony, either on the tips of the hyphae or directly in their path. Two days later it was observed that the growth of the colony had been retarded in the areas where the conidia had been added, so that noticeable bays were apparent around the circumference of the colony (Fig. 4). Microscopic examination revealed that many of the conidia had short germ tubes, and that diploidisation had occurred, as shown by the presence of clamp connections on the hyphae in the vicinity of the added conidia. This experiment was carried out with three pairs of compatible haploid mycelia, in triplicate, and in every plate hyphae with clamp connections were formed within 72 hr. of the addition of the conidia. Hence it was concluded that conidia borne on a haploid mycelium can germinate in the presence of hyphae and that the sporelings so produced are able to diploidise a suitable haploid mycelium.

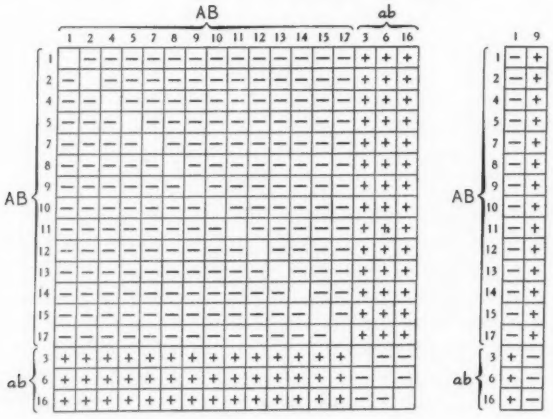
A second series of experiments was carried out to test the ability of conidia produced on diploid mycelium to diploidise haploid mycelia. Colonies of basidiospore culture No. 1 from No. *6503* (Fig. 5) were grown on malt extract agar in Petri dishes until they had reached a diameter of approximately 2.5 cm., and drops of a suspension of conidia from the diploid mycelium No. 1 × 9 were added at marked areas on the peripheries. Clamp connections appeared on hyphae of the original colonies in the vicinity of the added conidia within 72 hours. This was repeated with basidiospore culture No. 9 (Fig. 5). At this time the sporelings originating from the germination of the conidia were minute in size, so that the diploid mycelium that appeared could not have resulted from their germination, but only from the diploidisation of the original haploid colony. It seems evident that conidia produced on a diploid mycelium are capable of diploidising both the haploid mycelia that entered into the formation of the diploid mycelium.

In later experiments it was found that a few conidia germinated when sown on malt extract agar, the method of germination resembling that described for basidiospores. To evaluate the spores produced on a diploid thallus, conidia from the diploid mycelium No. 1 × 9 (Fig. 5) were sown on agar in Petri dishes, and although relatively few spores germinated, 17 single

conidium cultures were isolated. These produced haploid mycelia which were indistinguishable from those from single basidiospores. This verified the cytological observation that the conidia on diploid mycelium are uninucleate.

These 17 mycelia were grown together in pairs in all possible combinations and the resulting mycelia examined for the presence of clamp connections. The results are incorporated in Fig. 7, from which it may be observed that the mycelia fall into *two* groups, a member of one group reacting with a member of the other group in such a way as to produce mycelium bearing clamp connections.

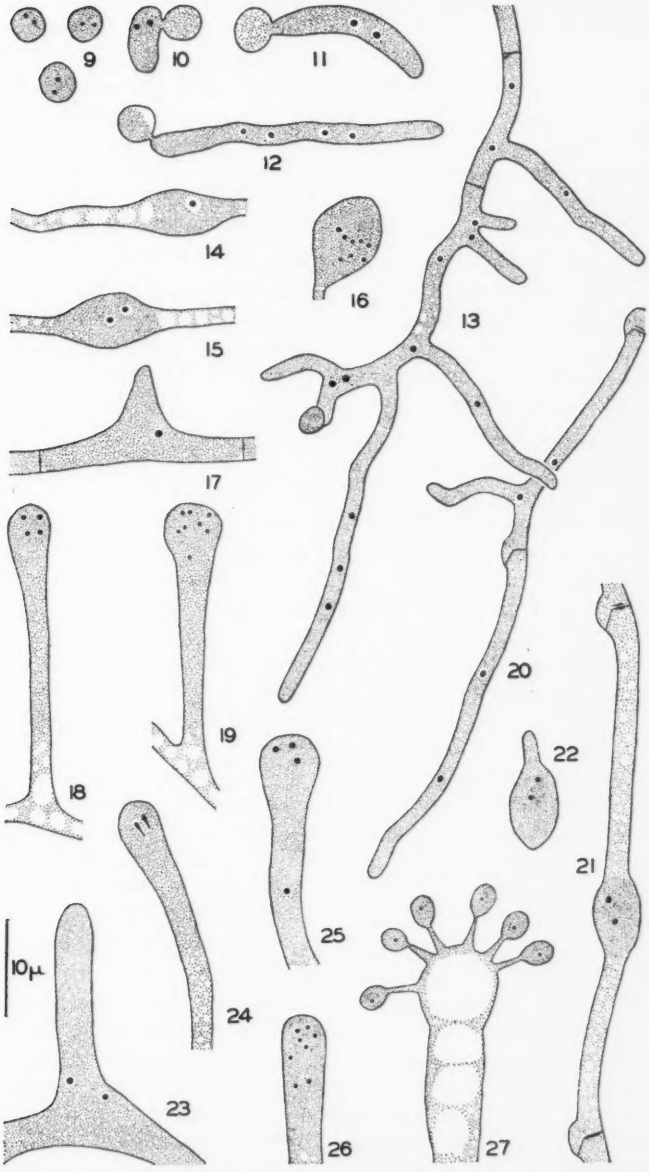
Each of the single conidium cultures was then paired with each of the parent basidiospore cultures, that is, 1 and 9 from Fig: 5, and the resulting mycelia examined for the presence of clamp connections. The results are summarized in Fig. 8 from which it is evident that single conidium cultures Nos. 1, 2, 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 17 form diploid mycelium when mated with basidiospore culture No. 9, and so belong to the group designated by the formula *AB* in Fig. 5 while conidium cultures Nos. 3, 6, and 16 react with basidiospore culture No. 1 and so belong to the group *ab*.



FIGS. 7 AND 8.

FIG. 7. *Corticium effuscatum. Results obtained by pairing in all possible combinations 17 mycelia, each derived from the germination of a single conidium produced on a diploid mycelium.* FIG. 8. *Corticium effuscatum. Results obtained by pairing the 17 mycelia of Fig. 7 with each of the parent mycelia.*

All the conidia produced on the diploid mycelium No. 1 × 9 are haploid in value and of only two types, as determined by their pairing reactions, and the two types are those of the two parent cultures. Hence, no segregation of factors, such as takes place in a basidium, occurred during the formation of the nuclei that entered the spores, that is, no fusion of the nuclei of the dikaryon preceded the nuclear divisions in the conidiophore. Thus the cytological observations recorded above are substantiated by the results of culture work.



FIGS. 9 to 27. *Corticium effuscatum.* FIG. 9. *Mature basidiospores.* ×*1400.* FIGS. 10 to 12. *Stages in germination of basidiospores.* ×*1400.* FIG. 13. *Young haploid mycelium.* ×*1000.* FIGS. 14 to 16. *Chlamydospores produced on haploid mycelium.* ×*1000.* FIGS. 17 to 19. *Stages in conidiophore development on haploid mycelium.* ×*1400.* FIG. 20. *Binucleate clamp-bearing hypha.* ×*1000.* FIG. 21. *Formation of chlamydospore in binucleate cell.* ×*1000.* FIG. 22. *Mature chlamydospore* FIGS. 23 to 27. *Stages in conidiophore development on diploid mycelium.* ×*3000.*

Discussion

Corticium effuscatum bears uninucleate conidia on oedocephaloid conidiophores on both haploid and diploid mycelia. While the production of oidia capable of repeating the haploid generation has been described for several species of Hymenomycetes, spores borne on organized conidiophores have been recorded for only a few species, including *Fomes annosus* (*Trametes radiciperda*) by Brefeld (4), *Peniophora Allescheri* by the author (12), *Pleurotus corticatus* by Kaufert (8), and *Corticium effuscatum* by Lyman (10).

Also, a few species are known in which uninucleate spores are produced on the dikaryotic mycelium. In most of the species this has been achieved by a reversion to the haploid condition of parts of the dikaryotic mycelium through the separation in a hypha of the nuclei of a dikaryon, and the subsequent growth of monokaryotic mycelium with the characteristics of such mycelium, including that of spore production. This was observed in cultures of *Corticium alutaceum*[1] by Lyman (10) and of *Collybia velutipes* by Brodie (6)

In *Pholiota aurivella*, Martens and Vandendries (11) observed that conidia borne on a diploid mycelium at first contain two nuclei which are later separated by a wall so that the mature spore is composed of two uninucleate cells. This may be interpreted as a return to the haploid condition within the spore. This haploid state may be very brief, ending with the disappearance of the septum and the germination of the spore, now binucleate, to give a dikaryotic mycelium; or the haploid condition may be continued as a result of the disjunction of the spore at the septum, with the germination of each uninucleate half to produce a haploid mycelium. Thus, in *Pholiota aurivella* there is a provision for a return to the haploid condition through structures borne on true diploid hyphae.

In *Peniophora Allescheri* described by the author (12) and in *Corticium effuscatum*, the conidiophores are borne on diploid mycelium and each originally contains a dikaryon, the nuclei of which divide simultaneously and successively until the head contains many nuclei, known to be of two kinds as to genetic constitution, but no longer existing in conjugate pairs. The subsequent migration of one nucleus into each conidium results in the formation of uninucleate spores on dikaryotic mycelium, a phenomenon rarely encountered in this group of fungi.

In *Corticium effuscatum* these conidia have lost to a marked extent their ability to germinate to give new individuals, at least under the conditions afforded them in the present investigation. Lyman (10) reached the same conclusion, writing (p. 181) "From his experiments the writer inclines to the belief that the conidia have partially lost the power of germination, and that they are not now of great importance in the reproduction of the plant". The present study has shown, however, that the conidia still retain an important

[1] *It is evident from Lyman's description and figures (10) that the species that he describes was one quite different from C. radiosum Fries (= C. alutaceum (Schrad.) Bres.). As suggested by Biggs (2) it is probable that Lyman was dealing with a form of C. coronilla v. Höhn. & Litsch.*

property in that they are able to bring about the diploidisation of suitable haploid mycelium.

Acknowledgments

The research here reported was conducted under the direction of Professor H. S. Jackson, of the Department of Botany, University of Toronto, to whom the author is indebted for stimulating suggestions and continued interest. She also wishes to thank Dr. R. F. Cain for supplying the specimens from which the cultures were obtained.

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POLARIZATION AND PROGRESSION IN PAIRING

I. INTERLOCKING OF BIVALENTS IN *TRILLIUM ERECTUM* L.[1]

BY STANLEY G. SMITH[2] AND E. ROGER BOOTHROYD[3]

Abstract

A total of 346 interlocks, occurring in five plants, were classified as to type of interlocking and type of bivalent concerned. Two main types of interlocking, true and false, occurred in equal numbers, the interlocking involving different bivalents and also the two arms of one bivalent. An interlocked chiasma, the first reported case in which the chromatids could be traced, and a clear example of chromatid interlocking were observed. No correlation was found between the frequency of interlocking and the temperature at which meiosis occurred.

The ratio of the frequencies with which the individual bivalents were involved in interlocking is proportional to the relative lengths of the bivalent arms minus a minimum length. Interlocking of bivalents in the 10 possible combinations in pairs occurred with the frequencies expected from the frequencies with which the individual bivalents were involved.

All loops of the bivalents were involved in interlocking, odd and even loops being involved in the ratio of 3 : 1. That adjacent to the centromere and the most distal loops were most frequently concerned and centric interlocking occurred with a frequency only one-third that expected on a random basis. The loops involved were larger than corresponding loops free from interlocking.

Interlocking was found to reduce the chiasma frequency. The chiasma frequency of encircled bivalents was higher than that of bivalents in cells devoid of interlocks, which, in turn, had a higher frequency than the encircling bivalents of false interlocks. The factor causing the reduction was found to reduce the chiasma frequency of non-interlocked bivalents accompanying interlocked bivalents. The reduction in chiasma frequency is insufficient to have resulted from loss of chiasmata. It is proportional to the increase in size of the interlocking loops, which is the result, not of increased repulsion, but of simple interference with the positions in which chiasmata are formed at pachytene.

It is concluded that interlocking is a result of the derangement of chromosomes that are normally polarized and have their proximal parts in an orderly spatial arrangement prior to pairing.

Introduction

In chromosome mutants of hexaploid wheats (*Triticum vulgare* Host) it was noticed (18) that in a number of cells two or more bivalents were interlocked at meiotic metaphase. The percentage of cells containing such interlocked bivalents and the number of bivalents interlocked in these cells is unusually high in a speltoid mutant having only 40 chromosomes instead of the usual 42. Other speltoid mutants which have either 41 or 42 chromosomes with a homozygous deficiency show progressively fewer cases of interlocking. The heterozygous speltoids, which have either 41 or 42 chromosomes (numerically and segmentally deficient respectively), have a still lower frequency of interlocking again both as regards percentage of cells and percentage of chromosomes (preliminary unpublished data of Mrs. E. R. Sansome). In the normal *T. vulgare*, interlocking is extremely rare.

[1] *Manuscript received March 24, 1942.*
Contribution from the Department of Genetics, McGill University, Montreal, Que.

[2] *Research Associate in McGill University.*

[3] *Holder of a Studentship under the National Research Council of Canada.*

Though a positive relationship clearly exists between degree of chromosome unbalance and frequency of interlocking, its cause could not be determined from observations at metaphase. But when during the making of smear preparations of *Trillium erectum* L. certain slides were found which contained a high percentage of interlocked bivalents, it appeared likely that their study might throw some light on the question of interlocking in wheat and the mechanics of chromosome pairing in general.

Triticum vulgare, besides being a hexaploid and possessing a large number of chromosomes which are individually indistinguishable, has chromosomes which it has not been possible to stain consistently so as to reveal their internal structure. *Trillium erectum*, on the other hand, is a diploid with only five pairs of chromosomes, each of which is morphologically distinct, and these can be readily stained to show the interrelationships of the four chromonemata of a bivalent. The present investigation was therefore undertaken in the hope of being able to discover some of the factors concerned with, and conditions resulting from, the interlocking of non-homologous bivalents.

Mechanics of Interlocking

As early as 1906 bivalent interlocking had been seen in *Salamandra maculosa* by Schreiner and Schreiner (see Belar, 3), and since that time it has been noted by many workers in a number of different organisms.

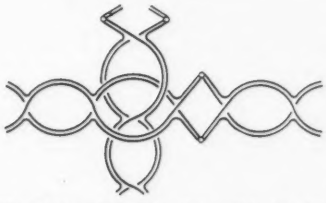


FIG. 1. *The relationship of the chromatids in a true interlock.*

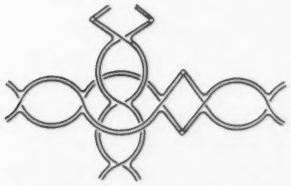


FIG. 2. *The relationship of the chromatids in a false interlock.*

When two homologues come together at zygotene, one or both members of another pair may be caught between them, resulting, with the formation of the necessary chiasmata, in "true" or "false" interlocking respectively (Figs. 1 and 2). Such interlocking has been seen at pachytene in *Dendrocoelum* (15), *Viviparous* (3), *Agapanthus* (9), *Dasyurus* (20), *Tulipa* (33), and in *Neodiprion* and *Chorthippus* (Smith, unpublished data). The interlocking of

non-homologous bivalents is therefore thought to be the result of a mechanical accident occurring during zygotene pairing (5, 14, 7, 23).

Dark (8) working with *Paeonia*, and Upcott (33) with *Tulipa*, found interlocking to be far more common in the tetraploid than in the diploid. One possible explanation given by Dark is that crowding of the chromosomes in the tetraploid would increase the chance of an interlock occurring. Upcott stresses the "large size of the nucleus" as well as "the large number of chromosomes present in the tetraploids", evidently implying that, relative to the diploid, there is need for more time in which to complete pairing.

Sax and Anderson (30, 31) suggest that bivalent interlocking may not be entirely mechanical in origin, but may be dependent upon the previous interchange of very short segments, the pairing of which would cause intertwining and subsequent interlocking of the chromosomes; these interchanges would be too small to be morphologically distinguishable. Dark (8) agrees that "true" interlocks occur at zygotene, but suggests that the "false" type may be the result of chromosome movements after zygotene. In *Eremurus spectabilis* Upcott (32) found false interlocking rare at metaphase compared with diplotene and diakinesis. She explains this as probably due to the bivalents slipping apart during metaphase orientation. Considering chromosome repulsion this seems more probable than that one should get pushed through another as Dark suggests.

In all the cases of bivalent interlocking which he noted in the tetraploid *Paeonia*, Dark found that the chromosomes were morphologically similar. This suggested to him that they might be homologous, and have been sufficiently closely associated to interlock either (*a*) through secondary pairing, or (*b*) through pachytene pairing to form a quadrivalent which did not persist, however, owing to failure to form the necessary chiasmata.

With regard to the position of interlocking, Mather (23) states that if pairing occurs at random along the chromosomes the position of interlocking should be random, and if there is little terminalization of chiasmata it should remain so. But should pairing start at the ends of the chromosomes interlocking would be most frequent in the central region and consequently if the centromere is median or submedian most of the interlocking would be in the centric loop. However, if interlocking has no influence on chiasma formation, as Mather (23) postulates, the position of interlocking would presumably be dependent solely on the chiasma frequency regardless of the method of pairing. At pachytene in *Agapanthus umbellatus*, Darlington (9) observed interlocking which usually coincided in position with the centromeres. This he suggested may result from pairing starting at the ends and pushing interlocks to the centromeres. The type of interlocking was difficult to ascertain, but from the absence of interlocks at metaphase most of it was thought to have been false. This supposition regarding the ultimate position assumed by interlocks is, however, not in agreement with most observations (Gelei *et al.*, see above); nor is that regarding the possible fate of false interlocks in agreement with our observations (see later, p. 376).

Catcheside (5), working with a mutant variety of *Oenothera Lamarkiana* having four bivalents and a ring of six chromosomes, found frequent interlocking of the bivalents both with the ring and with each other. He found that the relative chances of a bivalent interlocking with the ring or with another bivalent (3.13 : 1) are proportional to the relative lengths. The interlocking in this *Oenothera* appeared to occur at random between the bivalents. Like Darlington (9), Catcheside concludes that interlocking is a mechanical result of the pairing of the chromosomes commencing at the ends; a conclusion subsequently disproved by Marquardt (21, see later).

Cleland (6) and Catcheside (5) found two kinds of interlocked rings in *Oenothera*: two rings locked as links of a chain, and rings locked to form an apparent multiple chiasma. Interlocked links result when one chromosome passes between two others near their centromeres, chiasmata being formed on either side of the centromeres and distal to the interlock and subsequently terminalizing. An interlock of the apparent multiple chiasma type presumably results from one chromosome passing between two others in the distal region, with chiasmata formed on either side in that arm and subsequently terminalized. Without resolution of the chromatids, however, the true nature of this latter type is clearly open to question.

Marquardt (21) compared two varieties of *Oenothera*, *Oe. Hookeri* and *Oe. Hookeri flava sulfurea* (genetically *Oe. Hookeri*, but in *suaveolens* cytoplasm). In *Oe. Hookeri* at diakinesis, 99.08% of the bivalents were rings with two end-connections each, while in *Oe. Hookeri flava sulfurea* 5.86% were rods with only one end-connection. The difference between samples of 200 cells was more than seven times the standard error and hence significant. When compared with regard to interlocking, however, they were found to be the same (21.5 and 22.4% respectively). Marquardt suggested that this independence of interlocking and end-connection frequency might be due to interlocking occurring only in the central heterochromatic region of the chromosomes, while chiasmata are always formed distal to this region. Interlocked rings, which simulate multiple chiasmata, would, however, result only from interlocking having occurred in the euchromatic distal regions; although three of this type were observed by Catcheside (5) in 331 diakinesis nuclei, Marquardt failed to find any in his material.

Anderson and Sax (1) found that the percentage of interlocking varied greatly within species of *Tradescantia*, the variation between plants being from 0 to 27%. Finding no corresponding variation in chiasma frequency, although according to Sax and Anderson (31) "the correlation between chiasma frequency and the percentage of interlocking is positive", they state that this variation in the percentage of interlocking is due to minor environmental factors not affecting chiasma frequency. In *Eremurus spectabilis*, Upcott (32) found in different groups of cells a variation of from 0 to 15% of the cells containing interlocked bivalents. She suggests that such local variation in the amount of interlocking might be due to variations in the speed of early prophase stages: if rapid, the chances of homologues lying

together before pairing begins would be remote, and interlocking would be probable; if slow, on the other hand, the homologues would have time to approach each other before pairing actually starts, and the chances of interlocking occurring would be small. These two explanations of the similar variations in *Tradescantia* and *Eremurus* are compatible, since minor environmental factors, such as change in temperature, might affect the rate of prophase.

Interlocking and Chiasma Formation

That the configurations of interlocked bivalents afford evidence for distinguishing between the two main theories of chiasma formation has long been recognized.

Sax (29), quoting Gairdner and Darlington's (14) data, concluded that the great preponderance of proximal interlocking in *Campanula* argues in favour of the alternate opening out theory, or else shows that chiasmata pass off the ends of the chromosomes before metaphase.

In *Tradescantia*, Sax and Anderson (31) found that 99.4% of the interlocks were proximal, and a similar percentage was reported by Catcheside (5) in *Oenothera*. They considered this to be strong evidence in favour of the "classical" theory of chiasma formation. Later, however, they reconsidered the importance of this excess (1), pointing out that it may be due to the pairing being started at and restricted to the ends.

This possibility invalidates such evidence in favour of the "classical" theory. It should be stressed, however, that in forms such as *Tradescantia*, *Oenothera*, and *Campanula*, in which chiasmata are largely terminal by metaphase, a preponderance of proximal interlocking is obligatory.

Four cases of "double" or chiasma interlocking have been reported, where a loop in one bivalent is locked with two adjacent loops of another and around a chiasma between them. The first such case was reported by Mather (22) in *Lilium regale*, and one has since been seen by Upcott (32) in *Eremurus spectabilis*, by Beal (2) in *Lilium elegans*, and by Straub in *Gasteria strigosa* (See Oehlkers (26)).

These authors all agree that unless there had been two chiasmata in the centre, one of which had subsequently broken, the configuration could not have arisen by alternate opening out. Loss of a chiasma is unlikely unless interlocking causes chiasma breakage, since there is no reduction in the number of chiasmata from diplotene to metaphase in *Eremurus* and very little in *Lilium*. These materials, however, were mass stained and it would therefore be difficult, if not impossible, to distinguish between one chiasma and two very close together. Cases of chiasma interlocking are nevertheless strong evidence in favour of the partial chiasmatypy theory.

Mather (23) found no appreciable reduction in chiasma frequency in a limited number of interlocked bivalents in *Lilium* and claimed that this is to be expected on his assumption, since crossing-over would be unaffected by

interlocking at pachytene. Yet Gelei (15) and others have shown that the interlocked chromosomes interfere with the pairing of the interlocking chromosomes. Moreover, Dark (7) found only one chiasma in an interlocked bivalent instead of the two or three found in 48 other cases.

Gairdner and Darlington (14), Huskins and Smith (17), Dark (8), Upcott (32), and Darlington (12) all state that interlocked bivalent configurations were found which were explicable only on the partial chiasmatypy theory of chiasma formation, and not on the classical theory.

Interlocking and Terminalization of Chiasmata

Gairdner and Darlington (14) have stated that the occurrence of distal interlocking shows that terminalization had occurred without breaking of chiasmata; it cannot, therefore, be assumed that chiasmata ever break (or pass off the ends) during terminalization in the absence of this "exceptional strain" imposed by interlocked chromosomes. Sax and Anderson (31) obtained evidence from *Tradescantia* that interlocks are not released by chiasmata passing off the ends, and Marquardt (21) later showed that the frequency of end-connections in *Oenothera* is independent of the interlocking frequency.

According to Upcott (33) movement of chiasmata is slight in *Tulipa*. Where two bivalents each with one chiasma in each arm are interlocked, however, there is complete terminalization of the chiasmata, a condition never observed in free bivalents of this type. This throws further light on the mechanics of terminalization. Evidently the body repulsions of the chromosomes, increased by interlocking, reinforce their centric repulsions, and are strong enough to bring about terminalization of the chiasmata.

In *Pisum sativum*, Pellew and Sansome (27) found a configuration in which two pairs of segments in a segmental interchange ring of four were interlocked. The number of chiasmata was unusually high, 10 being observed, four more than had been observed in any other configuration. They suggest that interlocking might increase the number of chiasmata either by increasing the number of breaks in the chromatids at earlier stages or more probably by hindering terminalization. The former possibility is in disagreement with, among others, Dark's (7) observation on *Scilla* and Mather's (23) on *Lilium*. Since their configuration consisted of distal interlocking, and cancellation of interstitial chiasmata is apparently not proved, it may reasonably be assumed that distal interlocking would tend to hinder terminalization of the proximal chiasmata, whereas proximal interlocking would tend to aid in the terminalization of distal chiasmata.

General Appearance of Interlocked Bivalents

Mather (23) found in *Lilium* that when bivalents were interlocked the loops involved were generally enlarged. A similar condition was observed by Upcott (32) in *Eremurus spectabilis*. She also found that the two bivalents orientate themselves at right angles, as do successive loops of one bivalent, this obviously being the position of equilibrium. In the case of false interlocks both Mather

and Upcott state that the outer bivalent is always found to be around a chiasma in the inner one. Anywhere else the repulsion of the expanding arms of the encircled loop would tend to push the encircling loop toward one or the other chiasma. A single exception was found by Upcott, but the inner loop was not fully expanded. She suggests that in this case the outer bivalent must have been in the central equilibrium position, where the forces tending to push it towards one chiasma equalled those tending to push it towards the other. The appearance of these interlocked bivalents and the cases of terminalization of chiasmata noted by Upcott (33) in *Tulipa* offer further evidence of the non-specific nature of the repulsions between chromosomes.

As a result of interlocking the proper orientation of the bivalents on the metaphase plate is frequently upset (7, 8, 11, 12, 32).

Morgan, Bridges, and Sturtevant (25), Belling (4), and Sax (28) have suggested that interlocking may cause segmental interchange between non-homologous bivalents (translocation). Sax and Anderson (30) found an apparent correlation between interlocking and translocation in several species of *Tradescantia*, but admit that the interlocks might be the result rather than the cause. Finally, Koller (20) found in *Dasyurus* a configuration of two bivalents which indicated that they had been interlocked and then broken; the ends had rejoined in such a way that they would constitute a translocation.

Material and Methods

Rhizomes of *Trillium erectum* were collected at Ste. Agathe, Que., in September and October, 1937 and 1939. Slides were made from 10 rhizomes kept during meiosis in chambers maintained at various temperatures (Table I). At the appropriate time the anthers were removed, smeared, desiccated for

TABLE I

FREQUENCY OF INTERLOCKING

Slide	Temperature during meiosis	No. of cells examined	Cells with interlocks, %	Cells with true interlocks, %	Cells with false interlocks, %	Bivalents involved in interlocks, %	Interlocks*, %	
							True	False
66-*T*-89	3° – 4° C.	100	18	9	11	7.8	10	11
66-*T*-85	4° – 6° C.	200	33	18	17	14.2	19.5	20
66-*T*-81	8° – 10° C.	100	26	9	18	10.2	9	18
58-5*d*	16° C.	100	21	10	12	9.2	11	13
65-*M*-9	22° – 24° C.	200	56	39	31.5	29.4	54.5	43
Mean[1]			34.7	20.3	19.7	16.3	25.4	24
58-2*E*	4° C.	113	3.5	1.8	1.8	1.5		
58-3*D*	8° C.	100	17	9	9	3.6		
58-4*E*	11° C.	100	6	1	5	2.4		
58-5*G*	16° C.	100	13	7	7	2.8		
58-6*X*	20° C.	100	6	3	3	2.4		

* *See pp. 369 and 370.*

20 to 30 sec., and fixed in La Cour's 2BD fixative for about two hours. The slides were then washed in water, bleached in a solution of equal parts of hydrogen peroxide and 70% ethyl alcohol for one or two hours, and stained with crystal violet according to the method described by Huskins and Smith (17).

The "58" slides were similarly prepared by Dr. G. B. Wilson and Slide 65-*M*-9 by Dr. H. B. Newcombe.

Observations were made with a Zeiss 1.5 mm., 1.3 N.A. objective combined with 7×, 15×, and 20× oculars. Drawings were made with the camera lucida at original magnifications of 3875× and 5000×. Those reproduced herein bear no constant relationship to these original magnifications.

Observations

Two distinct lots of slides were examined (Table I). Those in the first were especially chosen because of the high frequency with which interlocking was observed; the second lot was selected later and examined only in order to determine whether or not there was a relationship between interlocking frequency and the temperature at which meiosis had occurred—a relationship implied by Upcott (32) but not substantiated by our observations on the first lot. All drawings, measurements, and other detailed analyses have been made from the slides comprising the first lot; the second will be omitted from considerations apart from the effect of temperature (see later).

General Appearance of Interlocked Bivalents

Early prophases were not examined for interlocking, but interlocked bivalents were observed at late diplotene, diakinesis, and metaphase. These interlocks were of two main types, termed in conformity with earlier workers, true interlocks (Fig. 1) and false interlocks (Fig. 2). Incomplete true interlocks were also found, in which one of the bivalents involved had failed to form the necessary distal chiasma. There is, therefore, the possibility that such interlocks might slip apart both before and during metaphase orientation of the bivalents on the equatorial plate. Likewise, since false interlocks are not held in place by chiasmata in both bivalents, it should be possible for them to slip apart before metaphase.

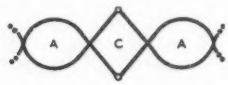
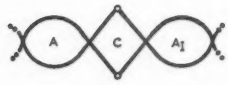
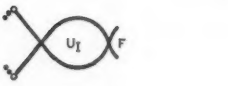
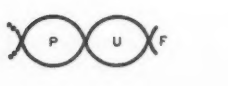
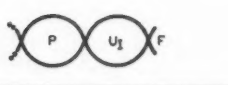
In the case of late diplotene interlocks the bivalents concerned show no definite orientation with regard to each other, frequently lying side by side with even the interlocked loops nearly parallel. By diakinesis, however, the bivalents generally lie at right angles to each other. In cases where the bodies of the bivalents lie nearly parallel, the interlocked loops, at least, tend to be almost at right angles to each other. This orientation of the loops appears to be an equilibrium position attained as a result of the mutual repulsion of the chromosomes.

In determining the effect of interlocking on loop size, comparisons have been made in two distinct categories. The first (Table II, 1 to 4) compares the size of the centric loop (*C*) with that of the loops (*A*) immediately adjacent to it and thus observations have necessarily been restricted to bivalents having

at least two chiasmata in each arm. The second (Table II, 5 to 8) compares the most distal, or ultimate loop (U) with the length of the free end (F) and,

TABLE II

EFFECT OF INTERLOCKING ON LOOP SIZE

Type of configuration	Relative size	Significance	Percentage length of bivalent involved
1 	*a.* $C > A = 33$ *b.* $C = A = 1$ *c.* $C < A = 62$	$\chi^2 = 8.8$; $P = <0.01$	$C = 13.0$ $A = 15.5$
2 	*a.* $C > A = 7$ *b.* $C < A = 13$ *c.* $C > A_I = 3$ *d.* $C < A_I = 17$ *e.* $A > A_I = 6$ *f.* $A < A_I = 14$	$\chi^2 = 1.8$; $P = <0.20$ $\chi^2 = 9.8$; $P = <0.01$ $\chi^2 = 3.2$; $P = >0.05$	$C = 14.1$ $A = 19.6$ $A_I = 25.9$
3	*a.* $C > A_I = 0$ *b.* $C < A_I = 4$		$C = 11.1$ $A_I = 32.9$
4	*a.* $C_I > A = 8$ *b.* $C_I < A = 0$		$C_I = 29.1$ $A = 15.6$
5	*a.* $U > 2F = 5$ *b.* $U < 2F = 17$	$\chi^2 = 6.6$; $P = 0.01$	$U = 20.2$ $F = 23.6$ $\frac{1}{2}C = 6.2$
6 	*a.* $U_I > 2F = 21$ *b.* $U_I < 2F = 10$	$\chi^2 = 3.8$; $P = 0.05$	$U_I = 33.8$ $F = 9.3$ $\frac{1}{2}C = 7.0$
7 	*a.* $U > 2F = 3$ *b.* $U < 2F = 6$ *c.* $U > P = 5$ *d.* $U < P = 4$ *e.* $P > 2F = 4$ *f.* $P < 2F = 5$		$U = 10.8$ $P = 15.0$ $F = 16.8$ $\frac{1}{2}C$ or $R = 7.4$
8 	*a.* $U_I > 2F = 21$ *b.* $U_I < 2F = 0$ *c.* $U_I > P = 19$ *d.* $U_I < P = 2$ *e.* $P > 2F = 15$ *f.* $P < 2F = 6$	$\chi^2 = 3.86$; $P = <0.05$	$U_I = 27.2$ $P = 13.2$ $F = 4.3$ $\frac{1}{2}C$ or $R = 5.3$

Note.—Mean percentage length of non-interlocked loops = 15.0.
Mean percentage length of interlocked loops = 29.5.
Mean of 2 + 3: $C = 13.6$.
$A_I = 27.1$.

in arms with three chiasmata, with the proximal or penultimate loop (*P*). The lengths were determined from camera lucida drawings and are expressed as percentages of the lengths of the chromosome.

In the absence of interlocking, *C* is somewhat smaller than *A* (Table II, 1). With an interlock in one of the *A* loops (A_1), that loop becomes larger than *C* in 17 out of the 20 cases measured (Table II, 2, *c* vs. *d*) while the other *A* loop, lacking an interlock, is larger only 13 times out of 20 (Table II, 2, *a* vs. *b*). The mean distance from the centromere to the chiasma on the interlocked side is 7.2, which is not significantly greater than the distance from the centromere to the chiasma on the non-interlocked side (6.9). The A_1 loops are also larger than the *A* loops in 14 out of the 20 cases. The mean percentage of *C* is considerably less than the mean of A_1 and, what is more remarkable, appreciably smaller than the mean of *A*. The *C* loop was found only twice with interlocks on both sides (Table II, 3); all these four A_1 loops were larger, with a mean of 32.9% against 11.1 for the *C*. It is worthy of note that the *C* mean, even with interlocks on both sides, is not appreciably smaller than when there is only one or none. Finally, on the eight occasions when the interlock involved the centric loop (C_1, Table II, 4), C_1 was always larger than the *A* loop, the mean of *A* in this case being approximately the same as when the bivalent is free from interlocking.

In the second analysis the ultimate loop (*U*) in two-chiasma arms free from interlocking was found to be *smaller* than twice the free end (2*F*) on 17 occasions out of 22, while, with interlocking in the loop (U_1), it was *greater* on 21 out of 31 (Table II, 5 vs. 6). In three-chiasma arms without interlocking (Table II, 7) the mean lengths of the ultimate and penultimate loops (*P*) were both less than that of the free end. Finally, with interlocking in the ultimate loop, however, *P* remains about as before, *F* is reduced to about ¼, but U_1 is increased to about two and one-half times (Table II, 8).

It should be noted in this connection that loops containing more than one interlock are considerably larger than the adjacent non-interlocking loops

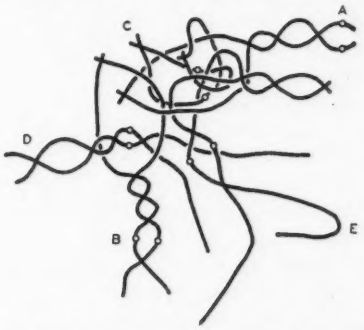


FIG. 3. *Simplified drawing of the most complicated cell analysed. False interlocks:— A encircling C and E, B encircling D. True interlocks:— A and B, A and C, C and E twice.*

(Fig. 3) and larger than loops containing only one (Fig. 5)—this is generally true.

Unlike the condition reported by Mather (23) in *Lilium*, and, with one exception, by Upcott (32) in *Eremurus*, where the outer bivalent of a false interlock is always around a chiasma in the inner one, in *Trillium* it is found that the outer bivalent is as frequently between chiasmata in the inner one as it is around a chiasma.

In cells containing interlocked bivalents the number of bivalents involved varied from one locked on itself to all five involved in interlocks. In Plant 65-*M*-9, where the frequency of interlocking was highest, one cell was seen with seven interlocks (Fig. 3), one with six, one with five, and five with four.

The three bivalents *C*, *D*, and *E*, with median or submedian centromeres (Fig. 4), were all found with their two arms locked together, the *E* 18 times, and the *C* and *D* five times each. The interlocks were true in 17 cases and false in 11. The subterminal *B* bivalent was also found locked on itself on one occasion, but the lock involved the long arm only; it was a true interlock.

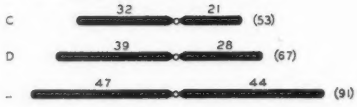


FIG. 4. *The relative lengths of the chromosomes and chromosome arms.*

Three instances were observed in which two bivalents were locked together in two separate places. In Plant 65-*M*-9, the *D* and *E* bivalents were locked in a true interlock, and one chromosome of the *D* also passed through another loop of the *E* in an incomplete true interlock. In another cell the *A* bivalent was locked around the *B*, and another loop of the *A* was also around one chromosome of the *B* forming an incomplete true interlock. In a third cell the *D* and *E* bivalents were doubly interlocked, there being a true interlock in both arms of each. In Fig. 3 the arms of the *C* have both formed true interlocks with one loop of the *E* and one is involved in a false and the other in a true interlock with one loop of the *A*. Finally, this same plant showed two cases in which the *E* bivalent was locked on itself and also with the *A*. In one, both the interlocks were true, in the other both were false. Three bivalents interlocked as links of a chain were found frequently in all plants except 66-*T*-81, in which only one out of the 100 cells analysed contained more than one interlock.

Only one case of chiasma interlocking occurred among the total of 346 interlocks found and this was in 65-*M*-9. One loop of the *B* bivalent was locked with two adjacent loops of the *D*, and around the chiasma between them (Fig. 5). It is noteworthy that this is the only case of chiasma interlocking so far reported in which the chromatids could be clearly traced. It

could be plainly seen that there was only one chiasma in the *D* bivalent between the pairs of chromatids of the *B*. The two arms of the *D* bivalent were also locked together in a true interlock, the loop in one arm being the same one as that locking the *B* chromosome.

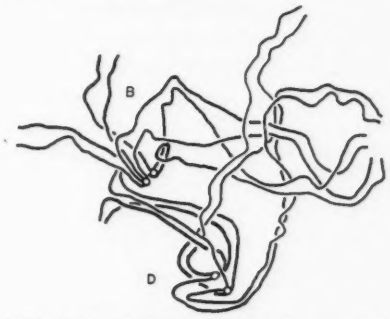


FIG. 5. *The B bivalent doubly interlocked around a chiasma in the D,—the two arms of the D are locked together.*

In 66-*T*-85 an example of chromatid interlocking was found (Fig. 6); in the loop next to the centric loop of the *E* bivalent one of its chromatids passed between the chromosomes of the *C*. If this chromatid had been outside the *C* instead of through it, the configuration would have been an ordinary false interlock of *E* around *C*. On the other hand, if the outer *E* chromatid had been alongside the inner one the configuration would have been a normal true interlock.

Frequency of Interlocking

Although interlocked bivalents have been seen in all *Trillium erectum* slides prepared in this laboratory, in the selected slides comprising the first of the

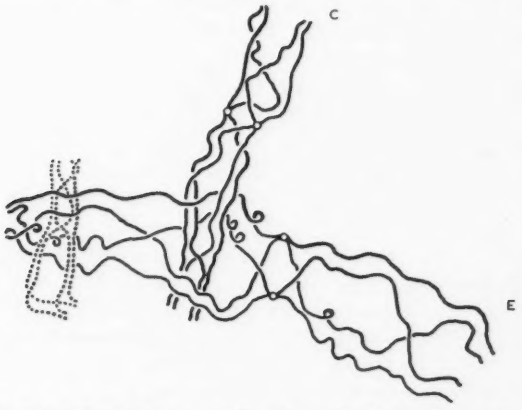


FIG. 6. *The C bivalent locked around one chromatid of the E (semidiagrammatic).*

two lots examined (Table I) the frequency of their interlocking was especially high. All percentages are based on counts of at least 100 cells—the actual numbers examined are given in Table I. The frequency of interlocking can be expressed in a number of ways. Here it is expressed as follows:— (1) the percentage of cells in which interlocking was observed, either true, false, or both combined, (2) the percentage of bivalents involved in interlocking, and (3) the number of interlocks, either true, false, or both, actually observed per 100 cells, i.e., the percentage of interlocking.

From 18 to 56% of the pollen mother cells in the first lot contained interlocked bivalents, the mean frequency being 34.7%. This is considerably higher than has been reported in most other materials, with the possible exception of ring-forming plants, such as *Oenothera*, in which 50% or more of the cells have been found to contain interlocks.

True interlocks were found in from 9 to 39% of the cells, the mean being 20.3%, while false interlocks varied in frequency from 11 to 31.5%, with a mean frequency of 19.7%. The ratio of cells containing true interlocks to those containing false interlocks (20.3 to 19.7) is approximately 1 : 1; the actual numbers of the two types of interlocking found (178 true and 168 false) are also approximately in the ratio of 1 : 1. The values of the true interlocks, both here and elsewhere, include, as well as ordinary true interlocks, any incomplete true interlocks found. These incomplete true interlocks constitute about 22.0% of the total number of true interlocks found.

The percentage of interlocks seen in the various plants ranged from 21 to 97.5 with a mean of 49.4%. In general, the percentages of true and false interlocks were not significantly different, but in plant 66-*T*-81 there were twice as many false as true. It is of interest to note that although plant 65-*M*-9 had interlocks in only 56% of the cells examined, when they are expressed as mean number of interlocks per cell, i.e., as percentage of interlocking, there is an average of almost one per cell. This is due to the relatively high frequency of cells containing more than one interlock found in this plant, as has been mentioned previously.

As shown in Table I the plants in the first lot underwent meiosis at temperatures ranging from 3° to 4° C. to 22° to 24° C. Plant 66-*T*-89, kept at 3° to 4° C., contained the least interlocking, regardless of how it is expressed, while plant 65-*M*-9, at 22° to 24° C., showed the highest value. These two values taken alone would seem to indicate a direct correlation between temperature and the amount of interlocking. Plants kept at intermediate temperatures, however, show a reverse relationship between temperature and interlocking. Plant 66-*T*-85, at 4° to 6° C., contained interlocked bivalents in 33% of the pollen mother cells, 66-*T*-81, at 8° to 10° C., in 26%, and 58-5*d*, at 16° C., in only 21%. It seems evident, therefore, that there is no simple correlation, either positive or negative, between the temperature at which the plants underwent meiosis and the amount of interlocking. To check whether or not the two opposite relationships shown were the result of some cause other

than direct temperature, the second lot of plants was studied. As is apparent from Table I, they showed that there is no direct relationship with temperature.

The respective frequencies with which the five bivalents were involved in interlocking were determined (Table III). These values were subdivided into four groups. The first, total interlocks, shows the frequency with which the bivalents were involved in interlocking of any kind. The second, true interlocks, shows the frequency with which they were involved in true interlocks. The third and fourth groups are composed of the bivalents involved in false interlocks, these being divided into the encircling and the encircled bivalents.

TABLE III

BIVALENTS INVOLVED IN INTERLOCKING

Slide	Total interlocks					True interlocks				
	A	*B*	*C*	*D*	*E*	*A*	*B*	*C*	*D*	*E*
66-*T*-81	12	10	1	8	23	4	3	0	2	9
66-*T*-85	35	33	9	23	58	19	15	4	10	30
66-*T*-89	6	8	2	9	17	2	6	1	4	7
65-*M*-9	77	71	52	64	126	38	45	29	37	69
58-5*d*	12	10	3	5	18	5	5	2	1	9
Total	142	132	67	109	242	68	74	36	54	124
Percentage	20.5	19.1	9.7	15.8	35.0	19.1	20.8	10.1	15.2	34.8

False interlocks

Slide	Encircling bivalents					Encircled bivalents				
	A	*B*	*C*	*D*	*E*	*A*	*B*	*C*	*D*	*E*
66-*T*-81	4	3	0	5	6	4	4	1	1	8
66-*T*-85	6	9	3	5	17	10	9	2	8	11
66-*T*-89	3	1	0	2	5	1	1	1	3	5
65-*M*-9	11	14	11	13	37	28	12	12	14	20
58-5*d*	4	2	0	1	6	3	3	1	3	3
Total	28	29	14	26	71	46	29	17	29	47
Percentage	16.7	17.3	8.3	15.5	42.3	27.4	17.3	10.1	17.3	28.0

Although the totals for true and false interlocks were almost the same, as were the relative frequencies of the five bivalents concerned in them, the relative frequencies of the encircling and encircled *A* and *E* bivalents in false interlocks were significantly different. The frequencies observed for individual plants are too low for their variations to have much significance, but in the case of the encircled bivalents of the false interlocks it is noteworthy that in Plant 65-*M*-9, which has the largest number of interlocks and in which variation should, therefore, be most significant, the value for the subterminal

A is 40% higher than that for the median *E*. Excluding Plant 65-*M*-9 from the totals for encircled bivalents the remaining values are *A*, 18 (22%), *B*, 17 (21%), *C*, 5 (6%), *D*, 15 (18%), and *E*, 27 (33%). These percentages correspond quite closely to those for the other groups, suggesting that some unusual condition, or conditions, in Plant 65-*M*-9 may be responsible for the difference between the encircled bivalent group and the encircling and true groups.

In an attempt to find whether this difference might not be due, in part at least, to special affinities between certain pairs of chromosomes, the false interlocks were analysed to determine the relationship between the encircling and the encircled bivalents (Table IV). Although the numbers are not very large it will be seen that the encircling *A* bivalents, whose frequency is only about half that of the encircled *A* bivalents, are encircling each of the different bivalents roughly half as often as the *A* bivalents are encircled by the corresponding pairs. Similarly the excess of encircling *E* bivalents is distributed among the other bivalents in a proportion fairly close to that in which the *E* bivalents are encircled by each of the other four bivalents. It therefore appears probable that the poor fit between the encircling and encircled bivalents is largely due to the *A* and *E* bivalents, which are inversely affected in the two categories. Further, the difference seems to lie mainly in Plant 65-*M*-9.

TABLE IV

FALSE INTERLOCKS

Encircled bivalents	Encircling bivalents					Total
	A	*B*	*C*	*D*	*E*	
A	*0*	12	5	7	22	46
B	6	*0*	3	4	16	29
C	5	5	*1*	0	6	17
D	3	3	2	*2*	19	29
E	14	9	3	13	*8*	47
Total	28	29	14	26	71	168

Bivalent Pairs

If Sax and Anderson (30) are correct in their suggestion that interlocking is the result of intertwining of the prophase threads owing to the interchange of very short chromosome segments, then it might be expected that certain bivalents would tend to be locked with each other in a disproportionately large number of cases.

In order to test this possibility the interlocks were analysed with respect to the pairs of bivalents involved (Table V). The false interlock values were taken regardless of which of the bivalents was the encircled and which the encircling one. The relative percentages of the 10 types of bivalent pairs

TABLE V

PAIRS OF BIVALENTS INVOLVED IN INTERLOCKING

Slide	*A–B*	*A–C*	*A–D*	*A–E*	*B–C*	*B–D*	*B–E*	*C–D*	*C–E*	*D–E*
66-*T*-81	4	0	2	6	0	1	5	0	1	5
66-*T*-85	9	2	6	18	1	6	17	0	6	11
66-*T*-89	0	1	0	5	1	2	5	0	0	7
65-*M*-9	22	9	11	34	8	13	25	6	19	26
58-5*d*	4	1	0	8	1	1	5	0	1	2
Total	39	13	19	71	11	23	57	6	27	51
Percentage	12.3	4.1	6.0	22.4	3.5	7.3	18.0	1.9	8.5	16.1
65-*M*-9										
Percentage	12.7	5.2	6.4	19.6	4.6	7.5	14.4	3.5	11.0	15.0

are markedly different. The *E* is involved in each of the three highest percentages and the *C* in the three lowest; since these are respectively long and short chromosomes it appears that a relationship exists between interlocking frequency and chromosome length. Such a relationship would be expected if interlocking is a chance phenomenon. The fact that not only all bivalents but all bivalent arms are involved, and then not always in the same positions, renders an explanation based on segmental interchange highly improbable.

The analysis was also carried out with the values for total interlocks broken down into true and false. The relative percentages of the 10 types of bivalent pairs are similar in true, false, and total interlocks, in spite of the very small numbers in certain categories. This similarity would seem to indicate that in this respect, unlike the individual bivalent values, the false interlocks behave in the same manner as the true ones. The cause of this similarity lies in the fact that the deficiency of encircling *A* bivalents relative to encircled *A* bivalents is compensated for by the deficiency of encircled *E*'s relative to encircling. In so cancelling each other out the total interlocks are similarly affected and the observed agreement between total, true, and false is, therefore, not unexpected.

Position of Interlocking

Earlier analyses of the position of interlocking have mainly been concerned with whether the interlock occurs in the centric loop ("proximal interlocking") or in some other loop ("distal interlocking"). As evidence for distinguishing between the classical and the partial chiasmatypy theories of chiasma formation the importance of the ratio of proximal to distal interlocking is slight, since it may be affected by other factors. More important is the consideration of whether interlocking occurs in odd- or even-numbered loops from the centromere.

In Table VI the position of interlocking is analysed. The loops are numbered outwards from the centromere, the centric loop being labelled 0, the

next loop 1, and so on, irrespective of which arms of the bivalents are involved. It should here be noted that since the centric loop can encircle a chromosome only so long as there is at least one chiasma in each arm and because the arms of the bivalents are scored separately, the frequency of centric interlocks given in Table VI is necessarily twice the true frequency.

TABLE VI

LOOPS INVOLVED IN INTERLOCKING

Plant	Number of chiasmata per arm																							
	1	2		3			4				5					1 to 6*								
	Loop involved																							
	0	0	1	0	1	2	0	1	2	3	0	1	2	3	4	0	1	2	3	4	5			
66-*T*-81	1	2	8	1	3	6	0	0	0	2	–	–	–	–	–	4	11	6	2	–	–			
66-*T*-85	4	0	36	0	9	9	0	1	0	2	–	–	–	–	–	4	46	9	2	–	–			
66-*T*-89	1	1	8	0	0	2	–	–	–	–	–	–	–	–	–	2	8	2	–	–	–			
58-5*d*	3	1	3	2	6	5	2	0	0	1	–	–	–	–	–	8	9	5	1	–	–			
65-*M*-9	10	2	36	5	22	10	3	11	2	5	1	2	1	1	3	22	71	14	6	3	–			
Total	19	6	91	8	40	32	5	12	2	10	1	2	1	1	3	40†	145	37	11	3	0			

* 65-*M*-9 *had two six-chiasma arms with the zero and second loops respectively involved.*

† *Note that the zero loop is actually involved only 20 times when the bivalent is considered as a whole.*

Of the 215 interlocks analysed 156 occur in the odd loops where, without interstitial loss of chiasmata, interlocking is impossible according to the classical theory. On the partial chiasmatypy theory, the distribution of interlocks into odd and even loops should, other things being equal, be dependent on the chiasma frequency. Taking into account the disparity in numbers of odd and even loops in the arms with uneven numbers of chiasmata, we find that instead of the proportion of odd to even being about 89 : 126, as it should on a random basis, it is 156 : 59. Clearly some factor or factors are influencing the distribution of the interlocks; what these may be will be discussed later.

Interlocking and Chiasma Frequency

In view of the interference in pairing resulting from interlocking, observed by many authors at pachytene, and the general increase in size of the interlocking loops seen at later stages in the present material, the working hypothesis that interlocking causes a reduction in chiasma frequency was tested. If this were so a progressive reduction should occur in bivalents of the following types:— (1) encircled bivalents in false interlocked pairs, (2) non-interlocked bivalents, (3) true-interlocked bivalents, and (4) encircling bivalents in false interlocked pairs. The encircled bivalent type in false interlocks was tentatively expected to have a higher chiasma frequency than non-interlocked bivalent because, in order to be encircled, chromosomes of this type must have been closely associated at the time of pairing.

We have determined the chiasma frequencies of the five different bivalents, *A* to *E*, in all five plants, grouping the four plants with the lowest interlocking frequency, and treating 65-*M*-9 separately since it has sufficiently high numbers in all categories and, incidentally, has the highest chiasma frequency. In the case of the four plants the analysis has been carried out for cells having no interlocks and for individual bivalents involved in true interlocks and the two types in false interlocks.

The data for the first four plants are given in Table VII and expressed as the mean chiasma frequencies of the individual bivalents, with a grouped mean for the set of five bivalents in each of the four categories. There are thus six lots of comparisons possible between individual bivalents of the five

TABLE VII

EFFECT OF INTERLOCKING ON CHIASMA FREQUENCY

Plant			*A*	*B*	*C*	*D*	*E*	Mean chiasmata per five bivalents
66-*T*-85, 66-*T*-81, 66-*T*-89, and 58-5*d*	False interlocks Encircled bivalents	No. chiasmata observed	19	30	15	21	45	
		No. bivalents observed	7	8	4	5	9	19.41
		Mean chiasmata per bivalent	2.71	3.75	3.75	4.20	5.00	
	Cells without interlocks	"	64	102	74	93	124	
			25	25	25	25	25	18.28
			2.56	4.08	2.96	3.72	4.96	
	True-interlocked bivalents	"	49	55	21	40	135	
			19	18	6	10	28	17.96
			2.58	3.06	3.50	4.00	4.82	
	False interlocks Encircling bivalents	"	29	25	8	31	55	
			11	7	3	7	12	17.89
			2.64	3.57	2.67	4.43	4.58	
65-*M*-9	False interlocks Encircled bivalents	No. chiasmata observed	40	35	22	16	45	
		No. bivalents observed	11	6	5	4	7	24.30
		Mean chiasmata per bivalent	3.64	5.83	4.40	4.00	6.43	
	Cells without interlocks	"	105	104	76	120	147	
			24	24	24	24	24	23.00
			4.38	4.33	3.17	5.00	6.12	
	Non-interlocked bivalents in cells with interlocks	"	110	110	129	149	108	
			30	27	39	32	17	22.06
			3.67	4.07	3.31	4.66	6.35	
	True-interlocked bivalents	"	45	56	35	54	128	
			14	14	9	11	22	21.82
			3.21	4.00	3.89	4.91	5.81	
	False interlocks Encircling bivalents	"	10	22	17	22	62	
			4	6	5	5	9	20.46
			2.50	3.67	3.40	4.40	6.89	

types, or a total of 30. When the comparisons are made according to the above hypothesis, it is found that observation fits expectation on 20 occasions, or in 67% of the cases. Further, it will be noticed that when the means are determined for sets of five bivalents, expectation is fulfilled in all four categories.

Similar data are also given in Table VII for Plant 65-*M*-9 with the addition of the mean chiasma frequencies of non-interlocked bivalents occurring in cells having interlocked bivalents. This analysis has been made to ascertain whether the conditions that reduce the number of chiasmata in interlocked bivalents also extend their influence to the accompanying non-interlocked bivalents. In the latter, potential interlocks may not materialize owing to a failure to form the necessary chiasmata. Here there are 50 possible comparisons of which 33, or 66%, were found to fit the hypothesis. Moreover, the mean chiasma frequency per set of five bivalents in the five categories shows the trend expected.

Discussion and Conclusions

Most of the data presented may be divided into two classes, (1) those concerned with the mechanics of interlocking, how interlocking occurs, and the forces governing the behaviour of interlocked bivalents, and (2) those that provide evidence concerning the method of pairing and chiasma formation. There is, of course, some overlapping between those two classes, since certain data have a bearing on both, but as far as possible they will be discussed separately.

Mechanics of Interlocking

The very variety of types of interlocking found indicates a certain randomness in their formation. All combinations of pairs of bivalents were found, true and false occurred in almost equal numbers, the two arms of bivalents with submedian centromeres were locked together, and the subterminal *B* was locked on itself. With the exception of the *B* bivalent, the remaining self-locked bivalents, 28 in all, were locked across the centric region, suggesting that the centromere is a point of prophase polarization. The observation of statistical equality of true and false interlocks (actually a slight excess of the latter) effectively refutes for *Trillium* both Dark's (8) suggestion that false interlocks may be due to postzygotene movement and Darlington's (9) and Upcott's (32) contention that false interlocks slip apart by metaphase.

(a) Bivalent Ratios

If interlocking is purely a matter of the chance relationship of the chromosomes to each other at the time of zygotene pairing, it seems reasonable to suppose that the ratio of the frequencies with which the five bivalents are involved in interlocks would be directly proportional to the total lengths of the chromosomes. To test this possibility the frequencies which would be expected for each bivalent were calculated, the lengths used being those shown in Fig. 4. These lengths were those determined by Huskins and Smith (17)

from measurements made at pollen grain mitotic anaphase. Since only relative lengths and not absolute lengths were needed for these calculations it was thought that they would be sufficiently accurate. In the case of the *A* bivalent the length used was that of the long arm only, since the centromere is so nearly terminal that it may be considered to be so in effect. The calculated values were compared with the values actually found (Table III), and χ^2 tests made to determine whether or not the values found fitted the hypothesis (Table VIII). The tests were made separately for the total interlocks, true interlocks, and the two types of bivalents in false interlocking.

As may be seen from Table VIII the value of χ^2 varied from 52.09 for the total down to 21.61 for the encircling bivalents of the false interlocks. With four degrees of freedom these all give probability values of considerably less than 0.01. It is obvious from this that the relative frequencies of the bivalents involved in interlocking do not fit the hypothesis.

TABLE VIII

BIVALENT RATIOS IN RELATION TO CHROMOSOME LENGTH

	A	*B*	*C*	*D*	*E*	χ^2	P $(N = 4)$
Total interlocks							
No. observed	142	132	67	109	242		
No. expected	104.7	136.7	113.2	143.1	194.4	52.086	<0.01
True interlocks							
No. observed	68	74	36	54	124		
No. expected	53.8	70.3	58.2	73.6	100.0	23.391	<0.01
False interlocks							
Encircling bivalents							
No. observed	28	29	14	26	71		
No. expected	25.4	33.2	27.5	34.7	47.2	21.606	<0.01
Encircled bivalents							
No. observed	46	29	17	29	47		
No. expected	25.4	33.2	27.5	34.7	47.2	22.184	<0.01

It was noticed that in the 132 cases in which the *B* bivalents were involved in interlocking the short arm was involved only once. This suggested the possibility that a minimum length of chromosome arm might be necessary for interlocking. If this were so it seemed reasonable to suppose that the short arm of the *B* must approximate very closely to this minimum length. On this assumption it is not the total length of the chromosomes which should be used to calculate the expected frequency of each bivalent, but the total length with the minimum value, 18, subtracted from each arm. Therefore, instead of 49, 64, 53, 67, and 91 for the *A*, *B*, *C*, *D*, and *E* bivalents respectively, 31, 28, 17, 31, and 55 should be used. In Table IX the frequency found for each bivalent is compared with the expected frequency calculated from the corrected lengths above.

The χ^2 values for the total interlocks, true interlocks, and encircling bivalents of the false interlocks on this basis were 6.76, 5.61, and 5.92 respectively.

TABLE IX

BIVALENT RATIO IN RELATION TO CHROMOSOME ARM LENGTHS MINUS A MINIMUM VALUE

	A	*B*	*C*	*D*	*E*	χ^2	P $(N = 4)$
Total interlocks							
No. observed	142	132	67	109	242		
No. expected	132.4	119.6	72.6	132.4	234.9	6.765	<0.10
True interlocks							
No. observed	68	74	36	54	124		
No. expected	68.1	61.5	37.4	68.1	120.9	5.609	<0.20
False interlocks							
Encircling bivalents							
No. observed	28	29	14	26	71		
No. expected	32.2	29.0	17.6	32.2	57.0	5.916	<0.20
Encircled bivalents							
No. observed	46	29	17	29	47		
No. expected	32.2	29.0	17.6	32.2	57.0	8.006	<0.05
Encircled bivalents excluding 65-*M*-9							
No. observed	18	17	5	15	27		
No. expected	15.7	14.2	8.6	15.7	27.9	2.101	>0.70

With four degrees of freedom these give values for P of less than 0.10, 0.20, and 0.20 respectively. These three types, therefore, all fit the minimum value hypothesis. In the case of the encircled bivalents of the false interlocks, however, χ^2 was 8.01, giving a P value of less than 0.05. It is due to the exceptionally high value for the *A* bivalent and the low value for the *E*, that this category does not fit the hypothesis as closely as the others do, but following the use of the corrected lengths there is a very substantial reduction in the χ^2 value, which is comparable to the reduction in the other three categories. It will be remembered that the encircled *A* bivalents of the false interlocks in Plant 65-*M*-9 occurred with a frequency 40% greater than that of the encircled *E* bivalents. When the 65-*M*-9 values for all five bivalents are omitted from the present analysis, the remainder fit the minimum value of hypothesis closely with a χ^2 of 2.10, giving $P = 0.70$.

From the results of the above tests it may be stated that, except possibly in the case of the encircled bivalents of false interlocks in 65-*M*-9, the frequency with which bivalents are involved in interlocks is proportional to the relative lengths of their arms minus a minimum length equal to that of the short arm of the *B* chromosome. This minimum length may be necessary for the formation of an interlock either because pairing is initiated at or near the centromeres, which are probably polarized (see later), or because it is the shortest length that will permit the formation of an interlock without inhibiting the formation of the distal chiasma necessary for its realization.

(b) Bivalent Pairs

Having considered the bivalents individually the next consideration is pairs of interlocked bivalents. Do the 10 types of bivalent pairs occur in the ratio expected from the frequency of interlocking of the individual biva-

lents? This would indicate randomness. Or do certain pairs occur with a frequency significantly greater than expected? This would indicate some specific attraction between the pairs such as might result from prior segmental interchange between them.

TABLE X

INTERLOCKING FREQUENCY OF PAIRS OF BIVALENTS

	A–B	*A–C*	*A–D*	*A–E*	*B–C*	*B–D*	*B–E*	*C–D*	*C–E*	*D–E*
Five plants										
Total observed	39	13	19	71	11	23	57	6	27	51
Total expected	37.83	16.58	28.81	59.94	15.18	26.37	54.87	11.56	24.06	41.79
	$\chi^2 = 12.92, N = 9, P = 0.20$									
True observed	21	3	9	35	3	16	32	4	18	19
True expected	19.91	7.74	13.27	28.76	8.20	14.05	30.45	5.46	11.84	20.30
	$\chi^2 = 13.02, N = 9, P = <0.20$									
False observed	18	10	10	36	8	7	25	2	9	32
False expected	17.75	8.88	15.61	31.22	6.96	12.23	24.47	6.12	12.24	21.52
	$\chi^2 = 15.75, N = 9, P = >0.10$									
65-*M*-9										
Total observed	22	9	11	34	8	13	25	6	19	26
Total expected	15.97	11.70	14.39	28.33	10.78	13.27	26.13	9.72	19.13	23.55
	$\chi^2 = 7.28, N = 9, P = 0.60$									

To answer these questions the frequencies expected for the various bivalent pairs were calculated from the frequencies of the individual bivalents excluding those locked on themselves. In Table X the frequencies found, separated into total, true, and false interlocks, are compared with the expected values, calculated from the frequency of interlocking of the individual bivalents as stated above. These three comparisons give χ^2 values of 12.92, 13.02, 15.17, which, with nine degrees of freedom, give values for P of 0.20, <0.20, and >0.10. Clearly, the frequencies observed correspond closely to the calculated ones. Since the objection might legitimately be raised that the use of the grouped totals from five unrelated plants would tend to minimize any inherent differences, the analysis was carried out separately for total interlocks in 65-*M*-9, the plant with the greatest incidence of interlocking and thus the one in which the test should be most significant. As is shown in Table X the value for χ^2 was 7.28, giving P equal to 0.60. It may be concluded from this that no specific attraction exists between any of the pairs of bivalents, the interlocking of these pairs being entirely random. This evidence is contrary to Sax and Anderson's proposal that interlocking may be the result of very short exchanged segments of chromosomes that cause the attraction and consequent interlocking of otherwise non-homologous chromosome pairs. It also renders improbable Dark's (8) suggestion that in tetraploids interlocking

is due to a failure to form the chiasmata necessary for quadrivalent formation among homologues closely associated either by secondary or pachytene pairing.

(c) Position of Interlocking

Interlocking was found in every internode of the bivalents except the fifth—there were, however, only two arms with as many as six chiasmata. The interlocks were particularly frequent in the proximal, i.e., the loop next to the centric loop, and the most distal loops, and occurred in them in about equal numbers. When these two coincide, i.e., in two-chiasma arms, the ratio of centric to proximal was 1 : 15, far lower than in any other case (Table VI).

If interlocking is a random phenomenon, the chance of an interlock arising in any particular loop should be dependent solely on the mean length of the loop. The one loop which is fixed in its position is, of course, the centric loop. Its mean length has been determined from measurements of 86 bivalents, each devoid of interlocking, and expressed as a percentage of the length of bivalent lying between the most distal chiasmata of the arms. The percentage of this length occupied by the centric loop was found to be 26.8 and on the assumption of randomness 26.8% of the interlocks in interlocked bivalents should therefore occur in the centric loop. Considering only bivalents with chiasmata in each arm the percentage of centric interlocks is, however, only 9.3 or one-third of the expected value (Table VI). This deficiency of centric interlocks would be expected if pairing were initiated at the centromeres and the proximal parts of the chromosomes were in an orderly spatial arrangement; it certainly does not indicate a fortuitous distribution at leptotene. Nor is it in agreement with the conclusion of many workers (5, 9, 24), that pairing starts at the distal ends.

(d) Orientation of Interlocked Bivalents

At late diplotene interlocked bivalents were found to take up no definite position with regard to each other. By late diakinesis or metaphase, however, they tend to be at right angles to one another as was noted by Upcott (32). This observation is in agreement with the generally accepted concept that by diakinesis there is developed a repulsion not only between the chromosomes of a bivalent but also between non-homologous chromosomes. It may reasonably be supposed that these are not two separate forces, but one non-specific repulsion. In the case of false interlocking bivalents both Mather (23) and Upcott (32) report that, with one exception noted by the latter, the encircling bivalent was always around a chiasma in the encircled bivalent. They hold that this is a natural result of the general repulsion operating between non-homologous bivalents. The absence of such a constant relationship in *Trillium erectum* is evidence that the force of repulsion in it is relatively weak, as is further instanced by the stable position of chiasmata, the rarity of terminalized chiasmata even when distal to an interlock, and the relative closeness of bivalents in general.

(e) Interlocking and Loop Size

It was noticed that the loops involved in interlocking were larger than the comparable ones free from interlocking on 69 occasions and smaller on only 14. The enlarged interlocking loops suggest two possibilities. The first, that the presence of another chromosome between a pair of homologous chromosomes may prevent the formation of a chiasma close to it; the second, that the development of the body repulsions of the chromosomes may cause the interlocking loops to become enlarged by the movement of chiasmata away from the interlock. Either of these possibilities might also explain the minimum length found necessary for interlocking. According to the first a subterminal interlock would be prevented by the inhibition of the distal chiasma necessary for its retention. According to the second the chiasma distal to the interlock might be forced off the end of the bivalent, thus allowing the two bivalents to slip apart.

That neither of these explanations is entirely adequate is shown by the following considerations. First, even in those bivalents having at least one chiasma in each arm, the frequency of centric interlocking is only about one-third of that expected. Second, the majority of interlocks occur in the most distal loop, where, if expansion caused by interlocking were sufficient to force a distal chiasma off, their frequency should be lowered. Admittedly, the penultimate loop would then assume the distal position and its interlocks would themselves become distal, but this would necessitate a direct correlation between interlocking and chiasma frequencies whereas the reverse has been found here. Further, it would demand a decreasing order of interlocking in the loops from the most distal to the most proximal, in order to fit the observation that, regardless of the stage of meiosis, the most distal loop has a high, if not the highest, frequency of interlocking.

A detailed analysis of Table II allows a choice between these two possibilities. With regard to the possible effect of repulsion on loop size, it is clear that if there is an effective repulsion the loop size can be changed by one or both of two movements—a shift of the proximal chiasma towards the centromere and a shift of the distal chiasma towards the end. That there is no movement *towards* the centromere in *Trillium* is clearly shown by Comparisons 1, 2, and 3 in Table II, where the *C* loop is of uniform size regardless of whether interlocks occur in one, both, or neither of the adjacent loops. This is shown even more clearly by the fact that in Category 2 the mean distance from the centromere to the proximal chiasma does not differ significantly in the presence or absence of an interlock (7.2 vs. 6.9 respectively). However, when an interlock occurs in the adjacent loop the overall length of the centric and adjacent loops is significantly greater than when there is none. Thus, grouping interlocks in Categories 2 and 3, we have a percentage length of 40.7 against 28.5 in Category 1. Since the centric loops are approximately equal, the size difference must be due to the relative positions of the distal chiasmata in the two cases. So far there is no conclusive evidence whether this is the result of simple interference with chiasma formation, or

of increased repulsion which is effective in moving chiasmata distally but ineffective in moving them proximally.

The fourth analysis supplies more definite evidence. In this case the proximal chiasmata are displaced distally by the presence of an interlock in the centric loop, leaving the centric loop about twice the size it is in the former analyses. Furthermore, the second chiasma completing the adjacent loop, is also displaced distally and displaced to such an extent that the loop, despite the "infringement" by the proximal chiasma, is nevertheless as large as when no interlock is present. This suggests that the increased size of interlocked loops is the result of simple mechanical interference and has no direct influence on the size of adjacent loops. This is illustrated beyond reasonable doubt in Table II where both $C + A_1$ and $C_1 + A$ occupy approximately 40% of the bivalent. Since the displacement due to interlocking occurs distally and not proximally, pairing in *T. erectum* must initially proceed from the centromere outwards along the arms.

Analyses 5 to 8 in Table II are in complete agreement with this conclusion and afford additional evidence on the time sequence of pairing. In two-chiasma arms the increase in size of loops involved in interlocking is gained at the expense of the distal portion, i.e., the free ends; thus $U + F = 20.2 + 23.6 = 43.8$ and $U_1 + F = 33.8 + 9.3 = 43.1$. The remaining part of the arm, R or $\frac{1}{2}C$, is therefore unaltered (6.2 vs. 7.0). Hence we conclude that in two-chiasma arms, or, on the average, in shorter arms, pairing starts at the centromere and moves outwards *to the ends*. Arms with three or more chiasmata are more variable distally and, consequently, the derived values will be less significant. One fact is established, however, and that is that little of the excess size of interlocked ultimate loops is gained at the expense of the more proximal loops; thus in 7 and 8, $P = 15.0$ and 13.2 respectively. It is gained largely from the free ends for their mean percentage lengths are 4.3 with interlocking and 16.8 without interlocking, a difference of 12.5 compared with a difference between the loops of 16.4. Part of the inequality is doubtless due to the variation in chiasma frequency in the arms comprising these categories, for this will cause differences in the length of bivalent occupied by the three distal chiasmata and the free end.

Frankel (13) has suggested that in the long arms of *Fritillaria* chromosomes there is, in addition to the primary centric pairing, a secondary pairing point located at the end of the chromosome. There appears to be no clear evidence of any such subsidiary pairing point being consistently operative here. Mather (23) has expressed the opinion that interlocking at pachytene would have no effect on chiasma frequency; this is contrary to the results obtained from the above analysis. Because a larger part of the bivalent is occupied by interlocking loops as compared with non-interlocking loops there should be a reduction in the chiasma frequency of interlocked bivalents (see later).

(f) Randomness of Bivalents Involved in Interlocks

The observations here made on *Trillium erectum* support the hypothesis, proposed by many workers, that bivalent interlocking results from a mechanical accident occurring during zygotene pairing. When homologous chromosomes pair one or both chromosomes of another pair may be caught between them. With the formation of the necessary chiasmata, these interlocks are retained as true and false interlocks, respectively. This theory would also explain the equal frequency of true and false interlocking, the random interlocking of the five bivalents in relation to the relative lengths of their arms minus a minimum length, and the random associations of pairs of bivalents in the ratio expected from their individual frequencies.

The exceptional behaviour of the *A* and *E* bivalents comprising the false interlocks in Plant 65-*M*-9 requires further consideration. Based on the grouped frequency with which the various bivalents were involved in true interlocks, there was a 34% deficiency of encircled *E* bivalents and a 24% excess of encircling *E* bivalents; in the case of the *A* there was a 70% excess of encircled bivalents and a 33% deficiency of encircling bivalents. Evidently some factor was acting to increase the chance of the *E* encircling another bivalent and to decrease its chance of being encircled. This factor, or a second one, was affecting the *A* bivalent in the reverse manner. Since this factor (or factors) must be operative at the time of pairing, it appears probable that the underlying cause may be a difference in linear homology resulting in the *E* chromosomes remaining apart longer and hence rendering them more liable to encircle a second bivalent. More or less complete homology of the *A* chromosomes would ensure their more immediate association, thus increasing their chance of encirclement by another bivalent and reducing the probability of their encircling another. In Plant 65-*M*-9 some evidence for a lack of linear homology was observed at anaphase in the form of dicentric chromatids, but unfortunately it was not possible to identify the bivalent involved.

Chiasma Formation

(a) Position of Interlocking

Interlocking was found in both odd and even loops, in every loop, in fact, apart from the fifth, from the centric loop to the most distal (Table VI). According to the classical theory of chiasma formation it should occur only in the even loops, except for occasional cases where a chiasma had broken. It was found, however, that, taking into account the disproportion of odd to even loops, the odd loops were interlocked three times as often as the even loops. This evidence is thus directly contrary to the classical theory.

Mather (23) has postulated that the position of interlocking is dependent on the method of pairing; that with pairing starting at the ends interlocking would be most frequent in the centric loop, while randomness in pairing would be associated with randomness in their spatial distribution. Apparently he follows Catcheside (5) and Darlington (9) in assuming that pairing progression ("zipper pairing") is uninterrupted by interlocked chromosomes which instead

are pushed towards the centromere. From the present study it appears, on the contrary, that the distribution of interlocks is dependent primarily on the spatial relationship of the threads at the time of pairing, and secondarily on the position in which chiasmata are subsequently formed.

(b) Interlocking and Chiasma Frequency

That a correlation exists between interlocking and chiasma frequency has been suggested by various workers (27, 7, 31), but has been denied by others (23, 1). The metaphase chiasma frequency could presumably be affected by interlocking in two ways, the one acting *after* chiasma formation to reduce the number originally formed, the other acting *before* either to decrease or to increase the number subsequently formed. The first possibility would necessitate the dissolution of terminal chiasmata, or the cancellation of adjacent interstitial free compensating pairs and elimination of one in adjacent interstitial continuous non-compensating pairs. Dissolution of terminal chiasmata appears to be ruled out by Marquardt's (21) demonstration that the frequency of end-unions in *Oenothera* is independent of the interlocking frequency. Cancellation of interstitial free pairs and elimination of one in continuous pairs remains a possibility, but would require an average reduction per interlocked bivalent of one and one-half chiasmata, since free and continuous pairs occur in approximate equality (16). Apart from Pellew and Sansome's (27) observation of an exceptionally high chiasma frequency in a self-interlocked ring of four in *Pisum sativum*, there appears to be no unequivocal evidence for the existence of a positive correlation such as would result from interlocking determining an increase in chromatid breakage (chiasma formation) at pachytene. Evidence for a negative correlation, however, is observational—the interlocked chromosome or chromosomes interfere mechanically with the association of the encircling pair at pachytene.

The hypothesis that interlocking causes a reduction in chiasma frequency has been established by the analysis of four plants combined and Plant 65-*M*-9 separately (Table VII). The five bivalents, *A* to *E*, in the five categories analysed in 65-*M*-9: (1) encircled bivalents in false interlocks, (2) bivalents in cells free from interlocking, (3) bivalents free from interlocking in cells having interlocked bivalents, (4) bivalents involved in true interlocks, and (5) encircling bivalents in false interlocks, have mean chiasma frequencies which when summated for each category are 24.30, 23.00, 22.06, 21.82, and 20.46, respectively. The question now arises, is the progressive reduction due to, (*a*) loss of terminal chiasmata which allows the slipping apart of interlocked bivalents, (*b*) cancellation or elimination of interstitial chiasmata, or (*c*) simple mechanical interference with the association of chromosomes at pachytene?

The first possibility can be dismissed immediately since the lowest chiasma frequency is not associated with the lowest frequency of interlocking. The second, cancellation and elimination of adjacent interstitial chiasmata, requires the opposite relationship, i.e., the relationship observed. As already mentioned, since free pairs constitute approximately half the chiasma

pairs formed and continuous pairs the majority of the remainder, this second possibility requires a reduction in chiasma frequency of 1.5 per bivalent (in bivalents with four or more chiasmata, Table XI) or 6.0 per set of five

TABLE XI

INTERLOCKING AND THE CANCELLATION OR ELIMINATION OF ADJACENT INTERSTITIAL CHIASMATA

Chromosome	Initial chiasma frequency (approx.)	Reduction with adjacent pair free	Reduction with adjacent pair continuous	Resulting chiasma frequency
C	3	0	0	3.0
A	4	2	1	2.5
B	4	2	1	2.5
D	5	2	1	3.5
E	6	2	1	4.5
Total	22	8	4	16.0

bivalents. The maximum difference observed, that between encircling and encircled bivalents of false interlocks, was only 3.84. If, on the other hand, the increased size of interlocked loops were the result of interference, the reduction in chiasma frequency would be proportional to the increase in loop size. Taking the increase in loop size as the mean of centric, adjacent, penultimate, and ultimate interlocked loops minus the mean for the corresponding loops free from interlocking, there is a total reduction of 14.5% in the length of bivalent remaining in which chiasmata can be formed. There should, therefore, be a similar percentage reduction in chiasma frequency between non-interlocked and interlocked bivalents. The mean chiasma frequency for non-interlocked bivalents in non-interlocked cells was 23.00 while the mean frequency for true and encircling bivalents was 21.14, giving an observed percentage reduction of 8.1. Now since at pachytene non-interlocked bivalents may have been potential interlocks that failed to materialize due to a failure to form the chiasmata necessary for their retention (see Categories 2 and 3 above), it may perhaps be justifiable to calculate the reduction on the basis of encircled rather than non-interlocking bivalents (for the reason given earlier). When this is done the observed percentage reduction in chiasma frequency, 13.0, fits expectation, 14.5, closely. We, therefore, conclude that the reduction in chiasma frequency is the result of interference rather than the cancellation and elimination of interstitial chiasmata.

(c) Chiasma Interlocking

The one case of chiasma interlocking found in Plant 65-*M*-9 is the first reported where the chromatids can be traced (Fig. 5). It is perfectly clear that only one chiasma is present in the *D* bivalent between the chromosomes of the *B*. Interpreted on the classical theory this would mean that sister chromatids were separated, passing on either side of one chromosome of the *B*,

in one loop, or that there had been two chiasmata in the *D* bivalent between the chromosomes of the *B*, one of these chiasmata subsequently having broken. The explanation on the partial chiasmatypy theory is far simpler and involves no abnormal condition; sister chromatids are paired in both the loops of the *D* bivalent concerned, and the chiasma arose as a concomitant of crossing over.

(d) Chromatid Interlocking

One case of chromatid interlocking was observed in Plant 66-*T*-85 (Fig. 6). Since all the chromatids could be traced there was no doubt that the *C* bivalent was locked around a single chromatid of the *E*. Gairdner and Darlington (14) state that chromatid interlocking should occur if the chromatids ever separate equationally. However, neither theory of chiasma formation, as then formulated, will explain the configuration seen. It could, of course, result from accidental breakage on either theory. If crossing over is conditioned by torsion, in the way suggested by Darlington (10, 12, p. 483), the explanation of the interlocking of a single chromatid would be the breaking and untwisting of one of the chromatids of the *E* (separated by the *C*) and its reunion with one of the chromatids of the homologue to form a chiasma on the other side of the *C* chromosome. The fact that the interlock as observed is some distance from the chiasma may be due to repulsion having shifted the *C* bivalent proximally relative to the *E* bivalent.

The evidence obtained from this study of interlocked bivalents in *Trillium erectum* strongly supports the partial chiasmatypy theory of chiasma formation, while no unequivocal evidence was found in favour of the classical theory.

Chromosome Orientation and Progression in Pairing

Based on the observation of (1) the relative frequency of interlocking between the two arms of a bivalent as opposed to interlocking within an arm, (2) the occurrence of centric interlocks with a frequency only one-third of that expected from the mean length of the non-interlocked centric loops, and (3) a minimum length determining the length–frequency relationship in all five bivalents, it is concluded that the centromeres are polarized, and that the proximal regions of homologues are in an orderly spatial arrangement. The centromeres, then, have an initial advantage in pairing. Being on the average more closely associated than are other regions of the chromosomes, the proximal parts will be first aligned for zygotene pairing, which will then progress, necessarily outwards, towards the ends of the arms. If, at the same time, other homologous regions lie sufficiently near one another (such as the ends, as a concomitant of centric polarization) pairing will be independently started and will progress, presumably, both proximally and distally. Non-homologous segments and interlocked chromosomes will, of course, cause a cessation of the centrically initiated progression. This may be overcome by two means, the one dependent on distal association of homologous parts, the other being a restoration of the earlier progression by relational twisting carrying the association across the non-homologous region of interference.

Unpublished data on wheat (18) show that both of these may fail. Chromosomes heterozygous for an interstitial deficiency fail in bivalent formation more often than when homozygous for the deficiency. Since the pairable length of chromosome is the same in both, the difference in bivalent frequency must result from the presence in the heteromorphic pair of the univalent segment. Further, although the frequency of bivalent failure is proportional to the length of the heterozygous deficiency, there is no corresponding failure when the deficiency is homozygous. Clearly, there would be no such lack of agreement if pairing started at both the centromere and the ends; Darlington's (12, p. 166) contention, based on Kihara and Nishiyama's (19) work on wheat hybrids, that pairing in *Triticum* starts at the ends is invalidated on other grounds by Huskins and Smith (18) and by Huskins, Sander, and Love (manuscript in preparation).

From the analysis of interlocking in relation to loop size it has been seen that an interlock has little or no influence on the position in which the proximal chiasma is formed, its main effect being to shift the distal chiasma towards the end. This may be interpreted as favouring relational coiling rather than distal contact as the means of re-establishing association, or that progression in pairing is slower towards the centromere than away from it. Finally, the fact that only one chiasma interlock was observed among the 346 interlocks found is strong evidence for polarization and also for mechanical interference consequent on interlocking.

Further evidence based on a study of somatic pairing in a dipterous parasite relates this phenomenon to polarization and the pairing of chromosomes at meiosis (Smith, in press).

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A NEW HEMIASCOMYCETE[1]

By W. Lawrence White[2]

Abstract

A new genus and species are established for a Hemiascomycete growing on *Corticium microsporum*, a member of the Thelephoraceae. The fungus is known from three collections made in different localities in Ontario. It is characterized by a limited mycelial growth in the subhymenium of the Basidiomycete where it also produces more or less elongate and coiled ascogonial branches and hypha-like antheridial branches, each female branch producing a single ascus which grows through the hymenium of the supporting fungus and protrudes prominently beyond the basidia. Phylogeny in the lower Ascomycetes is considered briefly and evidence is presented indicating relationship of the fungus in question with *Dipodascus* and *Endomyces*.

Introduction

In most classifications of the Ascomycetes there appears under the name used in the title above, a variously delimited and more or less controversial group of genera or families of morphologically simple forms. In its narrowest sense, i.e., in the sense of Varitchak (4), who is supported by Nannfeldt (3), it embraces only the genera *Dipodascus* and *Ascoidea*; in the broader sense of Gäumann (2, Hemiascomycetes, p. 137–165), it encompasses all those Ascomycetes, including the yeasts and the Taphrinales, that lack an ascocarp. The group in either sense, but more especially with respect to the genera *Dipodascus* and *Ascoidea*, has a peculiar interest and importance to all students of phylogeny in the fungi because of the prominent position accorded its members in any consideration of the origin of the Ascomycetes. Historically it consists of a miscellaneous accumulation of doubtful, misunderstood, and unrelated species; but the research and thought of the past 10 years have contributed much toward a better knowledge of some of the species and toward a better understanding of the group as a whole. Evidence is accumulating (1, 3, 5, 6) which tends to knit the Hemiascomycetes, even in the broader sense of the term, more firmly together than was formerly suspected. Attempts at a natural classification, however, still are hampered by a scarcity of connecting forms and by a paucity of detailed knowledge of certain known species, so that the discovery and assiduous study of additional forms is greatly to be desired. It follows then that the finding of a new non-ascocarpous form possessed of a combination of characters which is rather profoundly at variance with any hitherto known for the group—and which for this reason may in the future contribute toward an understanding of the group as a whole—warrants a more extended consideration than might be justified were it merely an addition to the number of known fungi.

[1] *Manuscript received May 14, 1942.*

Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, Cambridge, Mass., No. 210.

[2] *Bibliographer and Assistant Curator of Fungi, Farlow Herbarium.*

The fungus under consideration produces asci in the hymenium of *Corticium microsporum* (Karst.) Bourd. & Galz., where they occur scattered among the basidia, are much larger than the basidia, protrude prominently, and unless observed with extreme care would be interpreted as cystidia of the host, thus causing the latter to be referred to *Peniophora*. The ascomycetous nature of these cystidium-like bodies and the true identity of the supporting fungus were recognized recently by Dr. H. S. Jackson of the University of Toronto, while reworking a collection which had been taken in Ontario in 1937, and a portion of the material was communicated to the writer. The same thelephoraceous species was again found by Dr. Jackson to be bearing the Ascomycete in two collections made by him in Ontario, one in the summer of 1939 and the other in 1941.

Since Ascomycetes of which the morphology is of such a nature as to give any suggestion at all of identity with this one are so few in number of species, are so seldom encountered in nature, and have been given such prominence in the literature, it seems unlikely that the one at hand could previously have been placed on record and now overlooked. As it appears to be undescribed, and further since there is no known genus in which it may be placed, it becomes necessary to establish both genus and species for it.

Description

Helicogonium gen. nov. Ascocarpio nullo; mycelio vegetativo parco, hyphis angustis, ramosis, septatis, gerentibus et antheridia et acrocarpia (ramos ascogoniiferos); ramo femina laxe convoluto, septato, ad apicem cellulam tumidam ascogonialem gerente; ascogoniis singulis singularem ascum efformantibus; ascis elongatis, sessilibus, cum ascogonio continuis, apice incrassato, obtuso et, cum sporae deliberantur, dissoluto; ascosporis exiguis, hyalinis, bicellularibus, octo, at dein formantibus sporas secundarias numerosas intra ascum.

Ascocarp lacking; vegetative mycelium scanty, the hyphae narrow, branched, septate, producing both antheridial and ascogonial branches; antheridial branch consisting only of a septate hypha; female branch loosely coiled, septate, bearing a swollen ascogonial cell at its apex; ascogonia each extruding a single ascus; asci elongate, sessile, continuous with ascogonium, the apex thickened, obtuse, dissolving to liberate the spores; ascospores hyaline, two-celled, originally eight, budding within the ascus.

Helicogonium Jacksonii sp. nov. Ascocarpio nullo; fungo consistente e mycelio vegetativo crescente in subhymenio hospitis sui et producente ramos sexuales mares et feminas in eo his producentibus ascos; mycelio vegetativo parco, hyphis hyalinis, flexuosis, ramosis, septatis, tenui tunicatis, 1.8–2.5 μ in diam.; ramo mare consistente ex hypha tenuicula septata, haud manifeste distincta ab hyphis vegetativis, apice perficiente fusionem cum cellula ascogoniali; ramo femina brevi, laxe convoluto, incrassato ad apicem vel prope apicem, efformantem regionem ascogonialem, quae mox separatur septo; cellula ascogoniali subincrassata, curvata, ascum singularem ad latus convexum

efformante; ascis initio forma papillarum latarum gaudentibus, maturitate elongatis, forma nonnihil variabili, late clavatis, subcylindraceis vel subfusiformibus, 28–45 × 7.5–10 μ, subconstrictis ubi asci in ascogonia transeunt, sed ab his haud separatis dissepimento, apice obtuso-rotundato, 3–4 μ crasso, apparenter demum destructo actione solvente, incolorabili iodi ope; ascosporis hyalinis, oblongo-fusiformibus, 7–11 × 2.5–3 μ, bicellularibus, bi-seriatim vel multi-seriatim dispositis, gemmantibus dum in asco iam remaneant saepeque ad ultimam consumptis productione sporarum secundariarum; sporis secundariis formatis e sporis primariis gemmantibus ad apices suos, demum replentibus ascum, ellipsoideo-cylindraceis, 3–4.5 × 1.5–2 μ, duas guttulas olei minutas includentibus. Canada: Provincia Ontario. Ad *Corticium microsporum* . . . ad lignum acerosum.

Ascocarp lacking; fungus consisting of vegetative mycelium growing in the subhymenium of its host and producing there both male and female sex branches, the latter giving rise to asci; vegetative mycelium scanty, the hyphae hyaline, flexuous, branched, septate, thin-walled, 1.8–2.5 μ in diameter; male branch consisting of a slender, septate hypha not noticeably differentiated from the vegetative hyphae, fusing at its apex with an ascogonial cell; female branch short, loosely coiled, enlarged at or near the apex into an ascogonial region which is soon abjointed by a septum; ascogonial cell somewhat enlarged, curved, extruding a single ascus from the convex side; asci beginning as a broad papilla, elongating, at maturity somewhat variable in shape, broadly clavate, subcylindric, or subfusoid, 28–45 × 7.5–10 μ, slightly constricted at juncture with ascogonium but not separated from it by a cross wall, the apex obtuse, rounded, 3–4 μ thick, finally disappearing through solvent action, not coloured by iodine; ascospores hyaline, oblong-fusoid, 7–11 × 2.5–3 μ, two-celled, biseriate or multiseriate or irregularly arranged, budding while still in the ascus and often entirely consumed in the production of secondary spores; secondary spores formed by budding from the ends of the primary ones, finally filling the ascus, ellipsoid-cylindric, 3–4.5 × 1.5–2 μ, each containing two minute oil drops.

Canada: Province of Ontario: On *Corticium microsporum* (Karst.) Bourd. & Galz. on coniferous wood. Bear Island, Lake Temagami. Aug. 3, 1937. *A. J. Skolko. Type.* (In Farlow Herbarium and in University of Toronto Cryptogamic Herbarium No. *12919.*)—On *Corticium microsporum* on wood of *Pinus strobus* L., Petawawa Forest Reserve, Chalk River, Ont. Sept. 10, 1939. *H. S. Jackson.* (In Farlow Herbarium and in University of Toronto Cryptogamic Herbarium, *17569.*)—On *Corticium microsporum* on log of *Tsuga canadensis* (L.) Carr. Woods south of Aurora. Oct. 13, 1941. *H. S. Jackson.* (University of Toronto No. *17572*, but entire specimen deposited in Farlow Herbarium.)

There is no obvious evidence, either macroscopic or microscopic, that the Ascomycete is injurious to the *Corticium* upon which it grows. Superficially the hymenial surface of the supporting fungus appears normal, and under the microscope the asci are seen among the normally sporulating basidia. The

Corticium is delicate and fragile with a rather compact hymenial region supported by a broad substratal zone of erect, branching hyphae, very loosely arranged. The ascomycetous hyphae appear to be confined to the subhymenial layer of the *Corticium* and to be so scanty that they cause no noticeable increase in the compactness of the tissue of that region. Also the hyphae are so similar to the basidiomycetous hyphae among which they occur that it is difficult to trace them, and the exact relationship of the *Helicogonium* to the fungus upon which it grows has therefore not been determined. Very suggestive, however, are the short, slender lateral branches (Figs. 12, 14) which are seen frequently in crushed mounts; these seem to have a slightly specialized apex about which torn wall tissue adheres loosely, and it is possible that food and anchorage is obtained through the actual penetration of these apices into the basidiomycetous hyphae. It is certain that the ascomycetous hyphae do not reach the substratum of the supporting fungus and also that they do not even extend into the broad open substratal zone of the latter.

The fungus exhibits considerable simplicity, the scanty vegetative mycelium producing only antheridial and ascogonial branches (see illustrations), the latter each producing a single ascus. There is no ascocarp; ascogenous hyphae are lacking; and there is no evidence of a conidial stage. The antheridial branch is scarcely or not at all distinguishable from the vegetative mycelium except as it is later detectable by its apical union with the female branch, which appears as an elongate lateral appendage (Figs. 1, 2) that becomes approximately once loosely coiled and develops a terminal, or subterminal, thickened ascogonial region. An ascogonial cell (Figs. 3 to 7, etc.) is abjointed by the appearance of a septum at the stalk end of the thickened portion. A septum forms at the opposite end, at the point of union with the antheridium, following copulation. The whole process, in so far as can be determined from the dried material studied, is reminiscent of that illustrated for *Endomyces Magnusii* Ludw. (2, p. 144). In no case, however, has it been possible to trace the two copulatory branches to the same hypha.

All figures from type material mounted in potassium hydroxide–phloxine; drawn at a magnification of 1835 and reduced in reproduction to 1180.

FIGS. 1, 2. *Hyphae with coiled, unfertilized female branches.*

FIGS. 3 to 9, 11, 12. *Female branches to which male or antheridial branches have become attached.*

FIGS. 10, 13. *Immature asci.*

FIG. 14. *A more nearly mature ascus in which spore formation is in progress.*

FIGS. 15, 22. *Asci containing eight primary spores.*

FIGS. 16, 17. *Asci in which some of the eight primary spores have disappeared in the production of secondary ones.*

FIG. 18. *Ascus filled with secondary spores, the primary one having been entirely utilized in their production.*

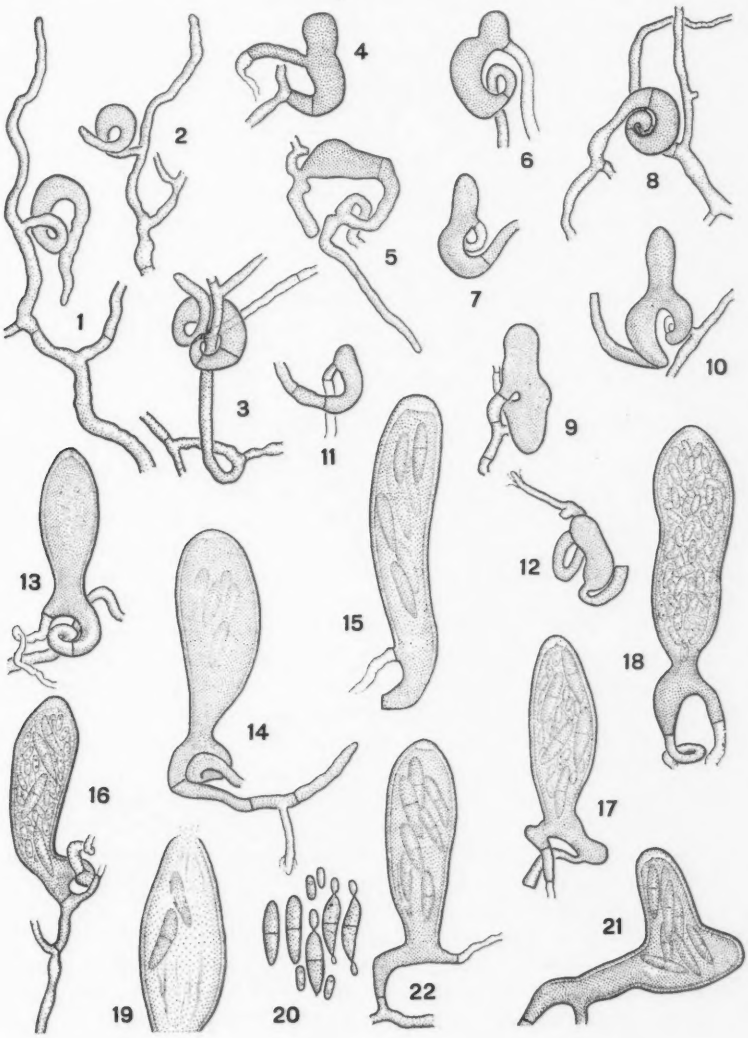
FIG. 19. *Ascus at time of spore discharge showing free passage at apex formed by dissolution of the wall.*

FIG. 20. *Primary and secondary ascospores.*

FIG. 21. *An abnormal ascus.*

For more detailed explanation, see text.

On the convex side of the inflated and somewhat curved ascogonium, and at a point furthest from the substratum, a rather broad and obtuse (Figs. 4, 5) bulge appears and elongates to form the single ascus (Fig. 10), which is merely a protrusion of the ascogonium. As the ascus elongates it broadens toward the apex (Fig. 13) so that at maturity there usually appears a slight constriction (Figs. 16, 18) at its juncture with the ascogonium, but there is no semblance of a stalk or pedicel and in no case is it cut off by a septum. The



ascus finally attains a size of 28–45 × 7.5–10 μ, and its shape (Figs. 16 to 19, 22) is in general cylindric though it varies to broadly clavate or more rarely subfusoid. As the ascus develops (Figs. 13, 14) the apex thickens (Figs. 14 to 17), especially just before or at spore formation, and becomes broad and rounded; later it gelatinizes to form a free passage (Fig. 19) for the spores. At no time is it coloured by iodine. Occasionally there appears to be some evidence of a pore; at least if an ascus is crushed before gelatinization of the apex begins, the contents are forced out through a pore-like opening, and also a suture in the wall of the apex (Figs. 16, 17) is very frequently in evidence. This thickening probably is to be compared with that described and illustrated by Biggs (1) for *Dipodascus uninucleatus*, rather than with that exhibited by the asci of the higher, inoperculate Discomycetes.

Spore formation, in so far as can be determined from material mounted in potassium hydroxide–phloxine, appears to be exactly as it is in the higher Ascomycetes, and there is no reason to suspect that it is different in any way. Eight elongate spores (Fig. 20) are formed in each ascus (Figs. 15, 22) and they mature basipetalously (Fig. 14). Almost immediately a septum appears in each spore, dividing it into two equal parts, and this is followed by the formation of secondary spores (Fig. 20) that are budded off from the original eight spores until they fill the ascus and leave the primary ones entirely exhausted (Fig. 18). Only rarely are asci found (Figs. 15, 21, 22) in which the eight original spores are present.

Phylogenetic Considerations

If one were to claim a derivation of *Helicogonium* from somewhere among the higher Ascomycetes he might find certain characters to support his contention: coiled ascogonium, reduced male element, elongate and cylindric type of ascus, elongate and septate spores with the usual number of eight per ascus before budding, and the character of the ascus tip which may be interpreted as not unlike that to be expected from retrogression in the typical explosive tip. However, there appears to be no strong evidence for the derivation of any of the non-ascocarpous forms from ancestors that produced a fruit body; the gap between forms having an ascocarp with its usually accompanying ascogenous mechanism and the simplicity of a form like that at present under consideration is too complete to permit the assumption of such retrogressive phyletic transition.

More generally accepted and more easily supported is the evolutionary series recently set forth in some detail by Nannfeldt (3) and fairly well embodied by him in a taxonomic outline. It presupposes *Spermophthora Gossypii* to be the nearest present-day representative of the antecedent stock which gave rise to three developmental series leading into the present higher Ascomycetes, and a fourth series—"absteigende"—leading through *Dipodascus*, *Ascoidea*, *Endomyces*, and finally to the yeasts. There is no claim that the series is monophyletic; and whether or not its prehistoric antecedents corresponded very closely with the present *Spermophthora* is not pertinent to the

correct placement of the fungus at hand. Because it lacks a fruit body or an organized hymenium and because of the immediate production of an ascus following plasmogamy—other characters not prohibiting—it may for taxonomic purposes at least, be placed in the aforementioned series of Nannfeldt nearest the forms to which it shows the greatest morphological likeness—perhaps near *Dipodascus uninucleatus* Biggs or *Endomyces Magnusii* Ludw.

Acknowledgments

The author is indebted to his colleagues, Dr. D. H. Linder, Dr. E. V. Seeler, and Dr. Rolf Singer for assistance rendered in the course of the preparation of this paper, and to Dr. H. S. Jackson of the University of Toronto for having made available the material on which the study was based.

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NORTH PACIFIC POLYCHAETA, CHIEFLY FROM THE WEST COAST OF VANCOUVER ISLAND, ALASKA, AND BERING SEA[1]

BY E. BERKELEY[2] AND C. BERKELEY[2]

Abstract

A list is given of 175 species of Polychaeta collected at the northern extremity of the west coast of North America; seven of the species are new to the entire coast, 29 to the northern extremity, and four new to science.

Notes are given on distribution and taxonomic characters of many of the known forms and the new species are described.

Introduction

The following five sources have contributed to the material on which this report is based:—

1. A collection made by Mr. E. G. Hart on the west coast of Vancouver Island when he was serving as biologist on the Canadian Hydrographical Survey boat *William J. Stewart* in 1934.
2. A collection made by Prof. C. McLean Fraser on the west coast of the Queen Charlotte Islands in 1935.
3. A small littoral collection made at Pacofi Bay on the east coast of the Queen Charlotte Islands by Mr. P. Parizeau in 1940.
4. A collection made by Mr. W. Williams of the yacht *Stranger*, owned by Capt. F. E. Lewis, when that vessel was cruising off the coast of Alaska, in the Bering Sea, and in adjacent Arctic waters, in 1937.
5. A few miscellaneous specimens taken by various collectors during the past few years in the region covered by the report and submitted to the authors for examination.

It seems advisable to report on these five collections together since (with the exception of a few specimens taken by Mr. Williams north of Bering Strait, which fall more nearly within the arctic area) they all originate in the same geographical region and contain a large number of species of Polychaeta in common.

Mr. Hart's material, which constitutes the greatest part of the combined collections, was all taken between Barclay Sound and Tatchu Point on the

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Contribution from the Pacific Biological Station, Nanaimo, B.C.

[2] *Voluntary Investigator.*

west coast of Vancouver Island. This stretch of coast was divided for purpose of record into four regions defined as follows:—

(*1*) Barclay Sound and the coast as far north as Amphitrite Point.
(*2*) Amphitrite Point to Estevan Point.
(*3*) Estevan Point to Bajo Point.
(*4*) Bajo Point to Tatchu Point.

The shore line and stations off the coast within lines running at right angles to the general direction of the coast line from the boundary points indicated are designated by the corresponding numbers in italics, (*1*), (*2*), (*3*), and (*4*), in recording Vancouver Island collecting localities in the report which follows. In addition to specimens taken between tide-marks, the collection comprises others taken both by trawl and by bottom sampler. The two latter classes are reported as "dredged" and the depth at which the specimens were taken is given for those in which it was recorded by the collector.

Prof. Fraser's collection was made on the west coast of the Queen Charlotte Islands between Houston-Stewart Channel and Rennell Sound. The majority of the specimens were taken between tide-marks, but a few taken by trawl, bottom sampler, or dredge are also in the collection. These also are reported as "dredged", but there is no record of depths. The precise locality at which each species was collected is given in the text.[1]

Mr. Williams' collection was submitted to the authors by Prof. G. E. MacGinitie, of the Kerckhoff Marine Laboratory, Corona del Mar, California. The data given by Mr. Williams of the conditions in which his specimens were taken is recorded.

Previous Work in the Region

Marenzeller (51) published a brief list of Polychaeta collected in Bering Sea by Dr. Krause in 1881. Moore (58, 59, 60) described the specimens collected by the U.S. Fisheries Steamer *Albatross* in 1903 at points throughout the region covered by the present communication. A number of specimens from Alaska are included in the report by Chamberlin (20) on the Polychaeta collected by the Canadian Arctic Expedition of 1913–18. Treadwell (73, 74) reported on the Polychaeta collected by Capt. R. A. Bartlett in Alaska in 1924. Bush (17) described tubicolous forms collected by the Harriman Alaska Expedition of 1899. A paper by the writers (10) contains an account of a few littoral species collected on the west coast of Vancouver Island in 1931. One of the writers has published a series of papers (4, 5, 6, 8, 9) dealing with the Polychaeta of the east coast of Vancouver Island. Although the polychaete fauna of this side of the island has characteristics differentiating it from that of the more exposed outer side and does not strictly come within the region defined as the subject of the present paper, it may be regarded as so doing in considering the wider aspects of the distribution of species. It will be so

[1] *Maps showing the regions covered by Mr. Hart's and Prof. Fraser's collections may be found in a paper by Dr. J. F. L. Hart on the reptant decapod Crustacea taken by the expeditions (Can. J. Research, 18(3) : 86-105. 1940.).*

regarded in the following pages. In this paper are mentioned, therefore, some hitherto unpublished records of species taken by the writers off the east coast of Vancouver Island. Finally, there are a number of records of Polychaeta from the region under consideration scattered through the literature; reference to these will be made in the appropriate places in the text.

A large number of the species dealt with here will be found mentioned in the literature cited, but many of the records are based upon a single specimen or very few. It has been found possible to amplify the descriptions and to extend the range of distribution of several of them.

List of Species

The following is a complete list of the 175 species herein recorded. The species marked (*) are new records from the west coast of North America. Those marked (**) have been previously recorded from the coast, but only from the region south of that covered by the present report, or from the Arctic. *Syllis stewarti*, *Pista moorei*, *Pista pacifica*, and *Scionella estevanica* are listed as new species. The type specimen of each of these species is in the author's collection.

APHRODITIDAE

Aphrodite japonica Marenzeller
Aphrodite negligens Moore

POLYNOIDAE

Lepidonotus coelorus Moore
Malmgrenia nigralba Berkeley
Gattayana cirrosa (Pallas)
Eunoe nodosa (Sars)**
Harmothoe imbricata (Linné)
Evarnella triannulata (Moore)
Arctonoe vittata (Grube)
Arctonoe pulchra (Johnson)
Arctonoe fragilis (Baird)
Polyeunoa tuta (Grube)
Lagisca multisetosa Moore
Enipo cirrata Treadwell
Halosydna brevisetosa Kinberg
Lepidametria longicirrata (Berkeley)

SIGALIONIDAE

Sthenelais verruculosa Johnson
Pholoe minuta Fabricius

ACOETIDAE

Peisidice aspera Johnson

AMPHINOMIDAE

Euphrosyne hortensis Moore

PHYLLODOCIDAE

Phyllodoce madeirensis Langerhans**
Phyllodoce groenlandica (Oersted)
Paranaitis polynoides (Moore)
Eulalia bilineata Johnston
Eulalia viridis (Müller)
Eulalia nigrimaculata Moore
Notophyllum imbricatum Moore
Eteone spetsbergensis Malmgren var. *pacifica*
Eteone barbata (Malmgren)**

TOMOPTERIDAE

Tomopteris septentrionalis Apstein

SYLLIDAE

Syllis heterochaeta Moore
Syllis harti Berkeley
Syllis armillaris (Müller)
Syllis sclerolaema Ehlers
Syllis fasciata Malmgren
Syllis elongata (Johnson)
Syllis stewarti sp. n.
Trypanosyllis gemmipara Johnson
Odontosyllis phosphorea Moore var. *nanaimoensis* Berkeley
Odontosyllis phosphorea Moore
Sphaerosyllis pirifera Claparède
Sphaerosyllis hystrix Claparède
Exogone lourei Berkeley
Exogone verrugera (Claparède)
Autolytus prismaticus (Fabricius)

NEREIDAE

Nereis cyclurus Harrington
Nereis pelagica Linné
Nereis vexillosa Grube
Nereis eakini Hartman**
Nereis callaona (Grube)
Nereis virens (Sars)
Nereis dumerilii Audouin and Milne-Edwards var. *agassizi* Ehlers

HESIONIDAE

Kefersteinia cirrata (Keferstein)

NEPHTHYDIDAE

Nephthys caeca Fabricius
Nephthys longosetosa Oersted**
Nephthys californiensis Hartman**
Nephthys caecoides Hartman
Nephthys ciliata (Müller)
Nephthys punctata Hartman**

GLYCERIDAE

Hemipodia borealis Johnson
Glycera robusta Ehlers**
Glycera capitata Oersted
Glycera americana Leidy
Glycera gigantea Quatrefages**
Glycera nana Johnson
Glycinde armigera Moore
Glycinde picta Berkeley
Goniada brunnea Treadwell
Goniada maculata Oersted**

EUNICIDAE (LEODICIDAE)

Eunice biannulata Moore**
Eunice kobiensis McIntosh
Onuphis elegans (Johnson)
Onuphis iridescens (Johnson)
Onuphis conchylega Sars**
Diopatra ornata Moore
Lumbrinereis latreilli (Audouin and Milne-Edwards)
Lumbrinereis brevicirra Schmarda**
Lumbrinereis bifurcata McIntosh**
Lumbrinereis inflata Moore
Lumbrinereis similabris Treadwell
Arabella iricolor (Montagu)
Drilonereis filum Claparède**
Ninoe gemmea Moore**
Dorvillea moniloceras (Moore)

ARICIIDAE

Scoloplos armiger (O. F. Müller)
Nainereis laevigata (Grube)

SPIONIDAE

Laonice cirrata (Sars)
Polydora caeca (Oersted)
Prionospio pinnata Ehlers
Prionospio cirrifera Wiren

CHAETOPTERIDAE

Phyllochaetopterus prolifica Potts
Mesochaetopterus taylori Potts
Telepsavus costarum Claparède
Chaetopterus variopedatus (Renier)**

CIRRATULIDAE

Cirratulus robustus Johnson
Cirratulus cirratus (O. F. Müller)**
Caulleriella viridis (Langerhans) var. *pacifica* Berkeley
Tharyx multifilis Moore**
Chaetozone setosa Malmgren**
Dodecaceria pacifica (Fewkes)

CHLORAEMIDAE

Stylarioides arenosa (Webster)*
Stylarioides plumosa (O. F. Müller)
Stylarioides papillata (Johnson)

OPHELIIDAE

Ophelia limacina (Rathke)**
Travisia forbesii Johnston
Travisia brevis Moore
Travisia pupa Moore

CAPITELLIDAE

Notomastus pallidior Chamberlin**
Eisigella tenuis (Moore)

ARENICOLIDAE

Arenicola pusilla Quatrefages

MALDANIDAE

Praxilella affinis (Sars) var. *pacifica* Berkeley
Maldanella robusta Moore
Nicomache lumbricalis (Fabricius)
Maldane glebifex Grube*
Maldane similis Moore
Maldane sarsi Malmgren
Notoproctus pacificus (Moore)
Petaloproctus tenuis (Théel)*

AMMOCHARIDAE

Myriochele heeri Malmgren
Ammochares fusiformis (Delle Chiaje)

SABELLARIIDAE

Idanthyrsus armatus Kinberg
Sabellaria cementarium Moore

STERNASPIDAE

Sternaspis fossor Stimpson

AMPHICTENIDAE

Pectinaria belgica (Pallas)
Pectinaria auricoma (Müller)?
Cistenides brevicoma (Johnson)
Cistenides granulata Linné**
Cistenides hyperborea Malmgren

AMPHARETIDAE

Ampharete goesi Malmgren**
Ampharete arctica Malmgren
Ampharete eupalea Chamberlin
Ampharete grubei Malmgren**
Amphicteis scaphobranchiata Moore
Amphicteis mucronata Moore
Sabellides octocirrata Sars*
Amage anops (Johnson)
Melinna cristata (Sars)
Lysippe labiata Malmgren**
Samytha sexcirrata (Sars)

TEREBELLIDAE

Amphitrite cirrata (O. F. Müller)
Neoamphitrite robusta (Johnson)
Terebella ehrenbergi Grube*

Pista cristata (Müller)
Pista fratrella Chamberlin
Pista pacifica sp. n.
Pista moorei sp. n.
Thelepus crispus Johnson**
Polycirrus kerguelensis (McIntosh)*
Polycirrus caliendrum Claparède
Terebellides stroemi Sars
Scionella estevanica sp. n.
Neoleprea spiralis (Johnson)
Artacama conifera Moore**

SABELLIDAE

Demonax leucaspis Kinberg
Eudistylia vancouveri (Kinberg)
Eudistylia polymorpha (Johnson)
Distylia rugosa Moore
Potamilla torelli Malmgren*
Pseudopotamilla reniformis (Leuckart)
Branchiomma burrardum Berkeley
Schizobranchia nobilis Bush
Schizobranchia insignis Bush
Fabricia sabella (Ehrenberg)
Chone gracilis Moore
Chone infundibuliformis Kröyer
Euchone analis (Kröyer)

SERPULIDAE

Serpula vermicularis Linné
Salmacina dysteri (Huxley)** var. *tribranchiata* (Moore)
Crucigera irregularis Bush
Crucigera zygophera (Johnson)
Chitinopoma groenlandica Mörch
Spirorbis racemosus Pixell
Spirorbis medius Pixell
Spirorbis ambilateralis Pixell
Spirorbis spirillum (Linné)

Family APHRODITIDAE

Aphrodite japonica Marenzeller (50, p. 111; 60, p. 338; 37, p. 21 (with synonymy))

Occurrence. Vancouver Island (*2*), dredged in 130 metres, and (*3*), dredged in 75 to 90 metres.

Aphrodite negligens Moore (58, p. 526; 4, p. 211)

Occurrence. Vancouver Island (*4*), littoral and dredged in 45 metres.

Remarks. Moore found no asperities on the notosetae of this species and suggested that this might be due to wear. The present examples show well defined asperities on all the notosetae. More usually they occur in the posterior region of the body only.

Family POLYNOIDAE

Lepidonotus coelorus Moore (56, p. 412; 34, p. 108 (with synonymy))

Occurrence. Cleveland Passage, Alaska.

Remarks. The elytral papillae are as Moore describes, unlike the more pointed ones figured by Hartman (34, Fig. 35*d*) and found by the writers in specimens from California.

Malmgrenia nigralba Berkeley (4, p. 213)

Occurrence. Vancouver Island (*4*), dredged in 45 metres.

Gattayana cirrosa (Pallas) (24, p. 49; 60, p. 337)

Occurrence. Humpback Bay, Alaska, dredged in about 15 fathoms, and Cleveland Passage, Alaska.

Remarks. This species has also been collected in Departure Bay, east coast of Vancouver Island in 15 fathoms.

Eunoe nodosa (Sars) (24, p. 51)

Occurrence. Arctic Ocean, 68° 50′ N.; 169° 20′ W. Dredged in 30 fathoms.

Harmothoe imbricata (Linné) (24, p. 55; 4, p. 215)

Occurrence. Vancouver Island (*3*), dredged in 137 metres, and (*4*), littoral; Western Skidegate Narrows, Queen Charlotte Islands, littoral; Pacofi Bay, Queen Charlotte Islands, littoral; Cleveland Passage, Alaska.

Evarnella triannulata (Moore)

Harmothoe triannulata (62, p. 346), *Evarne triannulata* (4, p. 215)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres.

Remarks. This species was originally dredged in considerable depths off the coast of southern California. Berkeley (4) records it from shallower waters off the east coast of Vancouver Island. It is interesting to note that in the more exposed waters of the outer coast of the island it is now again found in deep water. Chamberlin (19) points out that the generic name *Evarne* is preoccupied in Mollusca and substitutes *Evarnella*.

Arctonoe vittata (Grube) (34, p. 116; 37, p. 29 (with synonymy))

Occurrence. Vancouver Island (*2*), dredged in 77 and 172 metres, (*3*), dredged in 78 and 170 metres and littoral, and (*4*), littoral; Cleveland Passage, Alaska; Western Skidegate Narrows, Queen Charlotte Islands, littoral.

Arctonoe pulchra (Johnson) (34, p. 116)

Polynoe pulchra (41, p. 177), *Halosydna pulchra* (60, p. 329; 4, p. 212), *Lepidasthenia pulchra* (75, p. 144).

Occurrence. Vancouver Island (*2*), littoral, (*3*), dredged in 75 to 90 metres, and (*4*), dredged in 73 metres; Tassoo Harbour, Queen Charlotte Islands, dredged.

Remarks. Nearly all the specimens in this collection have the elytra mottled with brown, unlike those occurring on the east coast of Vancouver Island which usually have the pigment concentrated in a central area.

Arctonoe fragilis (Baird) (34, p. 116)

Polynoe fragilis (41, p. 179), *Halosydna fragilis* (4, p. 212), *Lepidasthenia fragilis* (75, p. 145).

Occurrence. Pacofi Bay, Queen Charlotte Islands, littoral.

Polyeunoa tuta (Grube)

Harmothoe tuta (42, p. 394), *Polynoe tuta* (60, p. 331; 4, p. 215)

Occurrence. Pacofi Bay, Queen Charlotte Islands, littoral.

Remarks. The elongated body with the elytra not confined to the anterior region justifies the classification of this species as above. A discussion of the genus *Polyeunoa* is given by Fauvel (28).

Lagisca multisetosa Moore (37, p. 54)

Occurrence. Vancouver Island (*2*), dredged in 45, 73, and 48.5 metres; Western Skidegate Narrows, Queen Charlotte Islands, littoral.

Enipo cirrata Treadwell (73, p. 1; 34, p. 120)

Occurrence. Pavlov Bay, Alaska.

Remarks. Commensal in the tube of *Nicomache lumbricalis* (Fabricius). Only the type specimen of this species has been known hitherto. This was also taken in Alaska. Hartman (34) has augmented the original description after re-examination of the type on which no elytra remain. The majority of the elytra are intact on the present specimen. They are small, suborbicular, smooth and glistening, have no marginal fringe, and are suffused with light brown pigment extending over about a third of the surface starting from the inner edge. They are well separated from one another leaving an area of the dorsum as wide as their diameter uncovered throughout the region of the body on which they occur. The distance between successive elytra varies from a complete diameter to a considerably smaller distance, but nowhere do they overlap. This separation of the elytra may be due in some measure to the flattened and extended condition of the specimen which is probably attributable to the close quarters in which it lives squeezed between the body and the tube of its maldanid host.

Halosydna brevisetosa Kinberg (37, p. 34 (with synonymy))

Occurrence. Vancouver Island (*2*), dredged in 77 metres and littoral, (*3*), littoral, and (*4*), littoral; Louscoone Bay and Skidegate Narrows, Queen Charlotte Islands, littoral.

Lepidametria longicirrata (Berkeley)

Lepidasthenia longicirrata (4, p. 214)

Occurrence. Vancouver Island (*4*), littoral.

Family SIGALIONIDAE

Sthenelais verruculosa Johnson (41, p. 187; 37, p. 62)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Pholoe minuta Fabricius (24, p. 120; 4, p. 216)

Occurrence. Pavlov Bay, Alaska.

Family ACOETIDAE

Peisidice aspera Johnson (41, p. 184; 4, p. 216)

Occurrence. Vancouver Island (*3*), dredged in 55 metres.

Family AMPHINOMIDAE

Euphrosyne hortensis Moore (58, p. 534; 4, p. 212)

Occurrence. Cleveland Passage, Alaska.

Family PHYLLODOCIDAE

Phyllodoce (Anaitides) madeirensis Langerhans (24, p. 150; 15, p. 28)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres.

Remarks. This species has been recorded by the authors from southern California (15). It was taken in that region in much shallower water. This and the present one seem to be the only records of the species from the west coast of North America.

Phyllodoce (Anaitides) groenlandica Oersted (24, p. 153; 5, p. 287)

Occurrence. Vancouver Island (*2*), dredged in 48.5 metres, and the following Alaska stations; Cleveland Passage, Nash Harbour, Nunivak Island (dredged in 8 to 10 fathoms), Spiriden Bay, Kodiak Island.

Paranaitis polynoides (Moore)

Anaitis polynoides (61, p. 339; 5, p. 287)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged.

Remarks. Southern (68) pointed out that the name *Anaitis* was preoccupied in Insecta.

Eulalia bilineata Johnston (24, p. 162; 5, p. 288)

Occurrence. Vancouver Island (*2*), littoral.

Eulalia viridis (Müller) (24, p. 160; 5, p. 288)

Occurrence. Vancouver Island (*2*), dredged in 73 metres, (*4*), littoral.

Eulalia nigrimaculata Moore (61, p. 344; 5, p. 288)

Genetyllis nigrimaculata (30, p. 119)

Occurrence. Vancouver Island (*3*), littoral.

Notophyllum imbricatum Moore (59, p. 217; 5, p. 287)

Occurrence. Vancouver Island (*4*), littoral.

Eteone spetsbergensis Malmgren var. *pacifica*

Eteone spetsbergensis (43, p. 102; 5, p. 289), *Eteone maculata* (72, p. 174 (non Oersted)), *Eteone pacifica* (30, p. 117)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. This form agrees with the stem species except in having a spotted dorsum. In this particular and in all others it agrees with Treadwell's *E. maculata.* It is noteworthy that Treadwell's figure of the first parapodium of this species is inverted. Hartman (30) points out that the name *maculata* is preoccupied and suggests *pacifica* as a specific name. The writers regard it as no more than a colour variety of *E. spetsbergensis.*

Eteone (Mysta) barbata (Malmgren) (24, p. 176)

Occurrence. Arctic Ocean, 68° 37′ N.; 168° 53′ W. Dredged in 30 fathoms.

Family TOMOPTERIDAE

Tomopteris septentrionalis Apstein (24, p. 224; 5, p. 289)

Occurrence. Queen Charlotte Islands. Plankton.

Family SYLLIDAE

Syllis (Ehlersia) heterochaeta Moore (61, p. 322; 13, p. 42)

Occurrence. Vancouver Island (*2*), dredged in 45 metres.

Syllis harti Berkeley (13, p. 35)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Syllis armillaris (Müller) (24, p. 264; 4, p. 206)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Syllis sclerolaema Ehlers (13, p. 40)

Occurrence. Vancouver Island (*4*), littoral.

Syllis fasciata Malmgren (49, p. 161; 15, p. 29)

Occurrence. Big Bay, Queen Charlotte Islands, littoral.

Remarks. This species is known from the east coast of Vancouver Island (authors' unpublished record) and has been reported (15) from Santa Cruz Island, southern California. Fauvel records it from Japan. It thus appears to be widely distributed in the North Pacific area.

Syllis elongata (Johnson) (4, p. 206; 13, p. 41)

Pionosyllis elongata (42, p. 403)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Syllis stewarti sp. n.

Occurrence. Vancouver Island (*2*), littoral.

Description

A single specimen 115 mm. long and about 1.5 mm. wide. The body is uniformly slender and has no definite markings. The dorsum, as preserved, is a uniform light brown. It is arched, so that the inconspicuous parapodia seem to be almost ventral.

The prostomium is about three times as wide as long. There are four reddish eyes about equal in size, the anterior pair placed only slightly in advance of the posterior pair. The palps are fused basally for more than half their length. The tentacles and tentacular cirri are moniliform and all are about the same length. The median tentacle arises between the posterior pair of eyes. The dorsal cirri are also moniliform and those of the first few anterior parapodia are longer than the tentacles and tentacular cirri and have about 12 articles, but they soon become shorter and for the greater part of the body are not much longer than the length of the segments and have only seven to nine articles. The ventral cirri are slender and taper gradually. The setae resemble those of *S. spenceri* especially those figured in the description of that species (13). There is no sign of budding.

This may be no more than a variety of *S. spenceri*, though it is very much longer and has not the characteristic dorsal markings of that species. *S. spenceri* is known only in the sexually active form. It is possible that the present specimen represents the asexual form.

Trypanosyllis gemmipara Johnson (42, p. 405; 13, p. 42)

Occurrence. Vancouver Island (*4*), littoral.

Odontosyllis phosphorea Moore var. *nanaimoensis* Berkeley (4, p. 207)

Occurrence. Canoe Passage, Skidegate Channel, Queen Charlotte Islands. Dip-net collection.

Remarks. As would be anticipated from the method of collection, all the specimens are in the epitokous form.

Odontosyllis phosphorea Moore (61, p. 327)

Occurrence. Ucluelet, Vancouver Island (collected by Dr. J. L. Hart). From old pile below the low-tide-mark.

Remarks. This specimen is in the atokous form. The epitokous form of the species has not yet been taken in the region though that of the variety *nanaimoensis* is common.

Sphaerosyllis pirifera Claparède (24, p. 301; 13, p. 43)

Occurrence. Vancouver Island (*4*), littoral.

Sphaerosyllis hystrix Claparède (24, p. 301; 13 p. 43)

Occurrence. Vancouver Island (*4*), littoral.

Exogone lourei Berkeley (13, p. 44)

Occurrence. Vancouver Island (*4*), littoral.

Exogone verrugera (Claparède) (24, p. 307; 13, p. 44)

Occurrence. Vancouver Island (*4*), littoral.

Autolytus prismaticus (Fabricius) (20, p. 12B; 13, p. 48)

Occurrence. Vancouver Island (*4*), littoral.

Family Nereidae

Nereis (Cheilonereis) cyclurus Harrington (29, p. 214; 39, p. 219)

Occurrence. Vancouver Island (*2*), (*3*), (*4*). All dredged in about 73 metres.

Remarks. This species was originally described as a commensal with hermit crabs living in gastropod shells. Moore (60) records cases of apparently free-living forms and Berkeley (5) confirms the suggestion that the species is not necessarily a commensal. In the present collection only one of the five specimens was accompanied by a gastropod shell in which, pre-

sumably, it had lived. The other four specimens seem to have been free-living. It is noteworthy that these four specimens have spotted parapodia, as described previously for a single individual by one of the writers (5), whilst the commensal does not show this character.

Nereis pelagica Linné (24, p. 336; 39, p. 225)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres; Skidegate Narrows, Queen Charlotte Islands, littoral; Cleveland Passage and Pavlov Bay, Alaska; Punuk Island, Bering Sea, dredged in 15 fathoms.

Remarks. The specimens from Pavlov Bay are in the heteronereid phase.

Nereis vexillosa Grube (21, p. 573; 5, p. 290)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), littoral; Masset Inlet, Queen Charlotte Islands (collector, Dr. A. Pritchard); Skidegate Narrows, Queen Charlotte Islands, littoral; Ketchikan and Humpback Bay, Alaska, littoral.

Nereis eakini Hartman (31, p. 472)

Occurrence. Vancouver Island (*4*), littoral; Big Bay and Skidegate Narrows, Queen Charlotte Islands, littoral.

Remarks. This species, previously recorded from California and Port Orchard, Washington, has also been taken by the authors on the east coast of Vancouver Island (unpublished record).

Nereis callaona (Grube) (23, p. 108; 39, p. 227 (with synonymy))

Occurrence. Vancouver Island (*3*), littoral.

Nereis virens (Sars) (24, p. 348; 5, p. 293)

Occurrence. Vancouver Island (*4*), littoral.

Platynereis dumerilii (Audouin & Milne-Edwards) var. *agassizi* Ehlers (54, p. 44)

Nereis agassizi (42, p. 399; 5, p. 292)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged; Skidegate Narrows, Queen Charlotte Islands, littoral.

Family Hesionidae

Kefersteinia cirrata (Keferstein) (24, p. 238)

Castalia fusca (44, p. 127; 4, p. 211)

Occurrence. Skidegate Narrows, Queen Charlotte Islands, littoral.

Remarks. This is a common shore form in the Nanaimo region, but, as it occurs there, it is small for the species. The present specimen is similar in all respects including that of size. McIntosh gives "tentacular cirri, two pairs". Actually there are eight pairs arranged in groups of 3, 3, and 2, as is described by Fauvel (assuming that the order of reading is from the setigers forward), but the two small, most ventral ones in the groups of three are very easily overlooked.

Family Nephthydidae

Nephthys caeca Fabricius (24, p. 365; 5, p. 290)

Occurrence. Vancouver Island (*4*); Pavlov Bay, Nash Harbour, Nunivak Island, Bering Sea, Humpback Bay, Alaska, all littoral.

Nephthys longosetosa Oersted (24, p. 367)

Occurrence. Vancouver Island (*2*), dredged in 17 and 50 metres.

Remarks. This species occurs at Balboa, Lower California (authors' unpublished record). Monro (52) lists it from the Panama region. It has not been recorded elsewhere on the west coast of North America. It is not to be confused with *N. longisetosa* Ehlers which is, according to Hartman (33, p. 146), a synonym of *N. magellanica* Augener and is recorded by her under that name from southern California.

Nephthys californiensis Hartman (33, p. 150)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Nephthys caecoides Hartman (33, p. 148)

Occurrence. Big Bay, Queen Charlotte Islands, dredged.

Nephthys ciliata (O. F. Müller) (24, p. 371; 60, p. 341; 5, p. 290)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged; Punuk Island, Bering Sea, dredged in 15 fathoms.

Nephthys punctata Hartman (33, p. 155)

Occurrence. Montagu Island, Alaska, dredged in 15 fathoms.

Remarks. This species has been taken off the east coast of Vancouver Island (authors' unpublished record).

Family GLYCERIDAE

Hemipodia borealis Johnson (42, p. 411; 39, p. 244)

Occurrence. Vancouver Island (*2*), dredged in 45 metres and littoral, (*4*), littoral.

Glycera robusta Ehlers (21, p. 656; 39, p. 246)

Occurrence. Vancouver Island (*3*), and (*4*), littoral; Houston Stewart Passage, Queen Charlotte Islands, littoral.

Remarks. These are the first records of this species north of California. One of the specimens is notable for the unusual development of the gill areas. These are strongly inflated and very prominent and occur on both the dorsal and ventral surfaces of the base of the foot. The writers recorded some very large specimens of the species from Elkhorn Slough, Monterey Bay, California, in 1935. Those from the Queen Charlotte Islands in the present collection are not as large as these, but are much larger than is usual.

Glycera capitata Oersted (24, p. 385; 6, p. 411)

Occurrence. Vancouver Island (*1*), dredged in 36 metres, (*2*), dredged in 172 metres, (*3*), dredged in 89 metres, and (*4*), littoral; Pavlov Bay, and Cleveland Passage, Alaska.

Glycera americana Leidy (21, p. 668; 39, p. 246 (with synonymy))

Occurrence. Vancouver Island (*2*) and (*3*), littoral, (*4*), littoral and dredged in 45 metres.

Glycera gigantea Quatrefages (24, p. 387)

Occurrence. Vancouver Island (*1*), dredged in 70 metres.

Remarks. The single specimen of this species seems to be a juvenile which, according to Fauvel, is the form described by Ehlers (21) as *G. lapidum*. No retractile branchiae can be made out. Ehlers says they are absent in *G. lapidum*. Monro (52) records *G. gigantea* from the Panama region and the writers (15) from southern California, but it does not seem to have been noted from more northerly latitudes.

Glycera nana Johnson (42, p. 411; 60, p. 347; 6, p. 411)

Occurrence. Vancouver Island (*2*), dredged in 17 metres, (*4*), dredged in 73 metres; Pacofi Bay, Queen Charlotte Islands, littoral; Cleveland Passage, Alaska.

Glycinde armigera Moore (63, p. 307; 39, p. 249 (*G. multidens*); 6, p. 411)

Occurrence. Vancouver Island (*1*), dredged in 36 metres, (*3*), dredged in 137 metres; Rennell Sound, Queen Charlotte Islands, dredged.

Glycinde picta Berkeley (6, p. 412)

Occurrence. Vancouver Island (*2*), dredged in 58 metres; Tassoo Harbour, Queen Charlotte Islands, dredged; Terror Bay, Kodiak Island, Alaska, Nash Harbour, Nunivak Island, Bering Sea, dredged in 8 to 10 fathoms, Cleveland Passage, Alaska.

Goniada brunnea Treadwell (70, p. 1174; 6, p. 412)

Occurrence. Vancouver Island (*1*), dredged in 36 metres.

Goniada maculata Oersted (24, p. 392; 39, p. 251)

Occurrence. Spiriden Bay, Kodiak Island, Alaska, dredged in 12 fathoms.

Family EUNICIDAE

Eunice biannulata Moore (57, p. 487; 38, p. 13)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. Hartman (35, p. 96) gives the distribution of this species "from Alaska south to San Diego, California". The writers know of no previous record of it north of California except a tentative one (6, p. 407) from the Nanaimo region. They believe the present to be the first record of a typical specimen of the species north of California.

Eunice kobiensis McIntosh (43, p. 278; 60, p. 345)

Occurrence. Vancouver Island (*3*), littoral; Houston Stewart Passage and Skidegate Narrows, Queen Charlotte Islands, both littoral; Cleveland Passage, Alaska.

Onuphis elegans (Johnson) (11, p. 771; 15, p. 35)

Northia elegans (42, p. 406)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. The anal end of this species has not been described. It is usually absent from collected specimens. Several such ends are in this collection. They carry four almost equal, very long, thin anal cirri.

Onuphis iridescens (Johnson) (15, p. 36)

Northia iridescens (42, p. 408; 60, p. 345)

Occurrence. Vancouver Island (*2*), dredged in 101 metres, (*3*), dredged in 89, 58, and 137 metres, (*4*), dredged in 45 to 55 metres; Cleveland Passage, Alaska.

Onuphis conchylega Sars (24, p. 415)

Occurrence. Cleveland Passage, Alaska.

Diopatra ornata Moore (63, p. 273; 6, p. 408)

Occurrence. Vancouver Island (*2*), dredged in 18, 58, 60, 73, and 137 metres.

Remarks. Considerable variations occur in the characters on which the diagnosis of this species is based. The writers have discussed these and the differentiation of the species from *D. californica* Moore elsewhere (15, p. 36). The tubes of the species are very variable, being constructed, apparently, of whatever is available to the animal for the purpose, stuck together on a membranous foundation. In most of the specimens in this collection the material consisted of broken tubes of *Telepsavus costarum* Claparède.

Lumbrinereis latreilli (Audouin and Milne-Edwards) (24, p. 431; 15, p. 38)

Occurrence. Vancouver Island (*1*), dredged in 70 metres; Tassoo Harbour, Queen Charlotte Islands, dredged.

Remarks. This is the form erroneously recorded by Berkeley (6, p. 409) as *L. zonata* Johnson (= *L. brevicirra* Schmarda) from the Nanaimo region. Monro (54, p. 84) lists it from Galapagos and Gorgona Island and the writers have recently encountered it in material from southern California.

Lumbrinereis brevicirra Schmarda (37, p. 161 (with synonymy))

Occurrence. Vancouver Island (*2*), (*3*), and (*4*), all littoral.

Remarks. *L. brevicirra* does not seem to have been recorded previously from the coast north of California.

Lumbrinereis bifurcata McIntosh (43, p. 241; 71, p. 196)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Lumbrinereis inflata Moore (63, p. 289; 32, p. 32 (synonymy))

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Lumbrinereis similabris Treadwell (74, p. 5)

Occurrence. Vancouver Island (*2*), dredged in 45 metres; Terror Bay, Kodiak Island, Alaska; Teller, Alaska, dredged in five fathoms; Pavlov Bay, Alaska; McLeod Harbour, Montagu Island, Alaska, dredged in 15 fathoms; Spiriden Bay, Kodiak Island, dredged in 12 fathoms, Humpback Bay, Alaska, dredged in 15 fathoms.

Remarks. This species was originally described from Bering Sea. It comes near to *L. impatiens* Claparède. It differs markedly in the shape of the posterior lobe of the foot in anterior segments. It is very deep, almost semicircular, in outline. In the median region the posterior lobes are normal in shape, both lobes are about the same length, and they project only slightly. Towards the caudal end the whole parapodium projects well and both lobes are somewhat elongated, the posterior ones being the longer. The acicula are black. Simple crotchets start at the seventh setiger. Evidently the species is common in the Alaskan region. The writers have also encountered it in material from Cadboro Bay at the southern end of Vancouver Island.

Arabella iricolor (Montagu) (24, p. 438; 10, p. 313)

Occurrence. Vancouver Island (*2*), and (*3*), littoral.

Drilonereis filum Claparède (24, p. 436; 54, p. 88)

Occurrence. Humpback Bay, Alaska, dredged in about 15 fathoms.

Ninoe gemmea Moore (63, p. 283)

Occurrence. Vancouver Island (*2*), dredged in 172 metres, (*3*), dredged in 137 metres.

Remarks. This species seems to have been recorded only from California. The writers have, however, collected it from Comox Spit, on the east coast of Vancouver Island.

Dorvillea (Stauronereis) moniloceras (Moore) (61, p. 256; 6, p. 410)

Occurrence. Vancouver Island (*3*), and (*4*), littoral.

Family ARICIIDAE

Scoloplos armiger (O. F. Müller) (25, p. 20)

Occurrence. Cleveland Passage, Alaska.

Remarks. A specimen of this species was wrongly recorded by the writers, from the west coast of Vancouver Island (10) as *Scoloplos elongata* Johnson.

Nainereis laevigata (Grube) (25, p. 22; 10, p. 313)

Nainereis robusta (61, p. 262), *Nainereis longa* (61, p. 264; 6, p. 413)

Occurrence. Vancouver Island (*3*), littoral; Pacofi Bay, Queen Charlotte Islands.

Family SPIONIDAE

Laonice cirrata (Sars) (25, p. 38; 12, p. 474)

Occurrence. Vancouver Island (*2*), dredged in 130 metres, (*3*), dredged in 137 and 89 metres; Big Bay, Queen Charlotte Islands, dredged.

Polydora caeca (Oersted) (25, p. 52; 12, p. 469)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Remarks. This species is common in the walls of the tubes of *Nicomache lumbricalis* (Fabricius) (= *N. carinata* Moore) from this locality. It does not seem to have been recorded elsewhere on the west coast of North America.

Prionospio pinnata Ehlers (23, p. 163; 26, p. 173; 15, p. 42)

Paraprionospio tribranchiata (6, p. 415), *Prionospio alata* (64, p. 185)

Occurrence. Vancouver Island (*4*), dredged in 74 metres.

Prionospio cirrifera Wiren (25, p. 62; 26, p. 174)

Prionospio multibranchiata (6, p. 414)

Occurrence. Spiriden Bay, Kodiak Island, Alaska, dredged in 12 fathoms.

Family CHAETOPTERIDAE

Phyllochaetopterus prolifica Potts (67, p. 972; 8, p. 307)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), dredged in depths varying from 18 to 555 metres.

Remarks. This species is known to occur over a large area and at a wide range of depths off the east coast of Vancouver Island. Evidently it is even more abundant off the west coast.

Potts described in some detail the process of asexual reproduction of this species by autotomy and regeneration, but did not observe any individuals bearing genital products. He surmised that this was probably due to insufficient examination rather than that the asexual method of reproduction had entirely replaced the sexual. The writers' observations confirm this. Mature individuals of both sexes have frequently been found in the tubes. They differ in no essential respect from the commoner, asexually reproduced, forms except in the abdominal region. This is both longer and stouter than in the asexual forms. There are usually some 30 to 40 segments which are so distended with sexual products that the animal is removed from its tube only with difficulty. The sexes are readily distinguishable by the colour given to the abdominal region by the ova and sperms respectively. The former are bright red-brown, the latter white.

Mesochaetopterus taylori Potts (67, p. 958; 8, p. 307)

Occurrence. Vancouver Island (*2*), dredged in 50 metres (tube only), (*4*), dredged in 45 metres and littoral.

Telepsavus costarum Claparède (25, p. 82; 54, p. 1052)

Telepsavus sp. (67, p. 969), *Leptochaetopterus pottsi* (7, p. 441)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), dredged in depths varying from 45 to 90 metres, and (*4*), littoral.

Remarks. This species was originally described as having only two body regions, but Berkeley's (7) observation of the occurrence of a third region in material from the east coast of Vancouver Island led to Monro's (54) re-examination of Claparède's species from Naples, in which also he found the third region. The specimens from the west coast of Vancouver Island agree exactly with those from the east coast except that the great majority are very considerably smaller. Those on the east coast frequently measure some 50 cm. after fixation, those from the west coast usually no more than 5 cm. This is probably associated with the fact that in the former the tubes are widely separated in the beds, whilst in the latter they are densely crowded. The species evidently occurs over a large area and in considerable beds, in many cases the dredge samples consisting almost entirely of its tubes. It seems to occur only rarely as a littoral form on the west coast, but is common between tide-marks on the east coast.

The writers commented in 1932 on the masses of empty chaetopterid tubes thrown up by the tide at Long Bay, on the west coast of Vancouver Island, but at that time could not identify the species. It is now clear that they consisted of the ends of tubes of *T. costarum* which doubtless had been broken off by the heavy seas prevailing along the coast.

Chaetopterus variopedatus (Renier) (25, p. 77)

Occurrence. Cleveland Passage, Alaska.

Remarks. Four specimens are small, but otherwise quite typical.

Family CIRRATULIDAE

Cirratulus robustus Johnson (42, p. 423; 8, p. 308)

Occurrence. Vancouver Island (*3*), (*4*), littoral.

Cirratulus cirratus (O. F. Müller) (25, p. 94; 20, p. 20B (synonymy))

Occurrence. Ketchikan, Alaska, littoral.

Caulleriella viridis (Langerhans) var. *pacifica* Berkeley (8, p. 307)

Occurrence. Skidegate Narrows, Queen Charlotte Islands, littoral.

Tharyx multifilis Moore (61, p. 267)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Remarks. The variety *parva* of this species occurs on the east coast of Vancouver Island (8, p. 307), but this is the first record of the stem species north of California.

Chaetozone setosa Malmgren (25, p. 101)

Occurrence. Vancouver Island (*3*), dredged in 58 metres.

Remarks. The only previous record of this species from the coast is from Friday Harbour, Washington (76).

Dodecaceria pacifica (Fewkes) (61, p. 268; 10, p. 314)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), all littoral.

Family CHLORAEMIDAE

Stylarioides arenosa (Webster) (45, p. 98)

Occurrence. Vancouver Island (*4*), dredged in 45 metres.

Remarks. Both *S. eruca* Claparède and *S. capulata* Moore have bidentate ventral crotchets similar to those occurring in the present species, but in both of them the barring of the shafts of these setae is much wider than in *S. arenosa*. Fauvel (25, p. 119) regards the latter species as a synonym of *S. eruca*. However, *S. arenosa* appears to differ, not only in the respect mentioned, but also in the tendency toward grooving of the dorsum and in the heavy firm coating of sand (making it feel like a hard, sandy rod), which McIntosh describes and which is quite characteristic. For these reasons the writers are inclined to doubt the suggested synonymy.

Stylarioides plumosa (O. F. Müller) (25, p. 116; 9, p. 69; 15, p. 47)

Occurrence. Pacofi Bay, Queen Charlotte Islands; Spiriden Bay, Kodiak Island, Alaska, dredged in 12 fathoms; Cleveland Passage, Alaska.

Stylarioides papillata (Johnson) (9, p. 69; 15, p. 46)

Trophonia papillata (42, p. 416)

Occurrence. Skidegate Narrows, Queen Charlotte Islands, littoral.

Family Opheliidae

Ophelia limacina (Rathke) (25, p. 132; 35, p. 107)

Occurrence. Nash Harbour, Nunivak Island, Alaska, dredged in 8 to 10 fathoms; Cleveland Passage, Alaska.

Travisia forbesii Johnston (45, p. 26; 25, p. 138; 60, p. 354)

Occurrence. Nunivak Island, Bering Sea; Cleveland Passage, Alaska.

Travisia brevis Moore (64, p. 220; 8, p. 313)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres; Humpback Bay, Alaska, dredged in about 15 fathoms.

Travisia pupa Moore (59, p. 228; 8, p. 313)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Family Capitellidae

Notomastus pallidior Chamberlin (18, p. 179)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Remarks. The specimens agree with the somewhat incomplete description given by Chamberlin. They show slight prolongations of the neuropodia which may function as branchiae.

Eisigella tenuis (Moore)

Notomastus tenuis (61, p. 277; 8, p. 312)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. The absence of both branchiae and genital pores in this species necessitates the above generic reclassification.

Family Arenicolidae

Arenicola pusilla Quatrefages (3, p. 114; 10, p. 315)

Arenicola claparedii (9, p. 68)

Occurrence. Vancouver Island (*3*), littoral.

Family Maldanidae

Praxilella affinis (Sars) var. *pacifica* Berkeley (8, p. 313)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Remarks. This variety has a heavy hook in each of the first three setigers closely resembling that figured for *P. gracilis* by Arwidsson (1, Pl. 9, Fig. 303) rather than that of the stem species. This variation was overlooked in the original description.

Maldanella robusta Moore (59, p. 236; 8, p. 314)

Occurrence. Tassoo Harbour, Queen Charlotte Islands, dredged.

Nicomache lumbricalis (Fabricius) (25, p. 190)

Nicomache carinata (59, p. 242)

Occurrence. Vancouver Island (*2*), and (*3*), dredged in 130 and 137 metres, respectively. Pavlov Bay and Cleveland Passage, Alaska.

Remarks. The large number of specimens of this species in the collection has enabled the writers to study the variation in the characters by which Moore differentiates it from *N. carinata*. As a result it is found that the only character on which a separation might be based is the colour of the first six segments. In this the specimens agree with the description given by Moore for *N. carinata*, but this fact does not seem to justify specific separation.

The sandy tubes are thick-walled, brittle, and closely coiled with a smooth lining throughout. Arwidsson (1, p. 91) describes similar tubes for *N. lumbricalis*.

The spionid *Polydora caeca* was frequently present in the walls of the tubes.

Maldane glebifex Grube (25, p. 199)

Occurrence. Vancouver Island (*3*), dredged in 89 metres.

Remarks. This species occurs widely on the east coast of Vancouver Island (authors' records), but has not been reported previously either there or elsewhere on the west coast of North America.

Maldane similis Moore (59, p. 233)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Remarks. The single specimen agrees closely with Moore's description except that it has one more chaetous segment. There are 19 such segments, segments 1 and 21 being achaetous.

Maldane sarsi Malmgren (25, p. 197; 60, p. 355; 64, p. 237)

Occurrence. Arctic ocean, 68° 37′ N.; 168° 53′ W., dredged in 30 fathoms.

Notoproctus pacificus (Moore) (2, p. 4; 8, p. 314)

Lumbriclymene pacifica (59, p. 246)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Petaloproctus tenuis (Théel) (1, p. 114)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Remarks. The species has not been reported previously from the coast, but a specimen recorded by Berkeley (8, p. 314) from the Nanaimo region as *Petaloproctus* sp.? is almost certainly Arwidsson's variety *borealis* (1, p. 118).

Family AMMOCHARIDAE

Genus *Myriochele* Malmgren. char. emend.

As *Myriochele* except that eyes may or may not be present.

Myriochele heeri Malmgren (25, p. 204; 9, p. 67)

Occurrence. Vancouver Island (*3*), and (*4*), dredged in 89 and 74 metres, respectively.

Remarks. The specimens in this collection all have the line of small pigment spots across the dorsal side of the prostomium and the larger, more heavily pigmented, circular spots at either end of the line described by Berkeley (9) in specimens from the Nanaimo region. Further investigation has shown that undoubtedly these are eyes. In all other respects agreement with *Myriochele heeri* is complete. The presence of eyes does not justify specific separation, but it makes necessary the above modification of the definition of the genus since Malmgren gives "Pars cephalica oculis . . . . . destituta".

Ammochares fusiformis (Delle Chiaje) (9, p. 67)

Owenia fusiformis (25, p. 203), *Ammochares occidentalis* (42, p. 420)

Occurrence. Vancouver Island (*4*), dredged in 74 metres.

Family SABELLARIIDAE

Idanthyrsus armatus Kinberg (40, p. 90; 54, p. 1066)

Pallasia armata (23, p. 195), *Pallasia sexungula* (22, p. 125)

Occurrence. Vancouver Island (*2*), dredged in 77 metres, (*3*), and (*4*), littoral. Humpback Bay and Cleveland Passage, Alaska.

Remarks. This is the species recorded by Berkeley (9, p. 74) from the Nanaimo region and wrongly assigned to *Pallasia johnstoni* McIntosh.

Sabellaria cementarium Moore (59, p. 248; 15, p. 50)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), dredged in 50 to 90 metres.

Family STERNASPIDAE

Sternaspis fossor Stimpson (69, p. 29; 62, p. 144; 9, p. 69)

Occurrence. Vancouver Island (*3*), dredged in 137 to 180 metres. Arctic Ocean, 68° 37′ N.; 168° 53′ W., dredged in 30 fathoms.

Family AMPHICTENIDAE

Pectinaria belgica (Pallas) (25, p. 220; 15, p. 51)

Occurrence. Vancouver Island (*1*), dredged in 36 metres, (*3*), dredged in 137 metres. Rennell Sound, Queen Charlotte Islands, dredged. Terror Bay, Kodiak Island, Alaska.

Remarks. The specimens in this collection have the almost smooth edge to the dorsal plate and the characteristic caudal region which differentiate the species from *P. auricoma* (Müller).

Pectinaria auricoma (Müller)? (25, p. 222)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Remarks. The single specimen is attributed as above with some doubt since, although the dorsal plate has the toothed edge which characterizes the species, the caudal region resembles that of *P. belgica* (Pallas).

Cistenides brevicoma (Johnson) (8, p. 311)

Pectinaria brevicoma (42, p. 423)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Cistenides granulata (Linné) (48, p. 359; 20, p. 25B)

Occurrence. Point Hope, Alaska; Nash Harbour, Nunivak Island, Alaska, in 8 to 10 fathoms; Humpback Bay and Cleveland Passage, Alaska.

Remarks. The species is distinguished by the small number (seven or eight on each side) of blunt paleae, fewer than recorded for *C. brevicoma* (Johnson), which species it resembles in many respects. The uncini differ in that there are only three large teeth in the present species whilst those of *C. brevicoma* have four or five. The common occurrence of typical specimens of *C. granulata* in Alaskan waters seems to bear out the suggestion made by one of the writers (8, p. 311) that a transition occurs between these two species along the west coast of North America as the habitat becomes more southerly. If this be the case they should be regarded as one species.

Cistenides hyperborea Malmgren (48, p. 360; 71, p. 212)

Occurrence. Terror Bay, Kodiak Island, Alaska.

Remarks. This species has 11 to 12 paleae on each side. Those that are unworn have long, slender, pointed tips. The uncini have four large teeth. In this latter respect the specimen agrees with the Japanese form of the species as recorded by Nilsson (see 65, p. 57).

Family AMPHARETIDAE

Ampharete goesi Malmgren (48, p. 364)

Occurrence. Vancouver Island (*1*), dredged in 36 metres.

Remarks. The species is distinguished by the shape and number of the paleae and the arrangement of the branchiae in a straight line across the dorsum. It has not been recorded previously from the west coast of North America, but is a not uncommon arctic form.

Ampharete arctica Malmgren (48, p. 364; 15, p. 51)

Occurrence. Vancouver Island (*2*), (*3*), dredged in 50 and 95 metres, respectively.

Ampharete eupalea Chamberlin (20, p. 25B)

Ampharete seribranchiata (74, p. 7)

Occurrence. Arctic Ocean, 68° 37′ N.; 168° 53′ W., dredged in 30 fathoms.

Ampharete grubei Malmgren (48, p. 363)

Occurrence. Cleveland Passage, Alaska.

Amphicteis scaphobranchiata Moore (59, p. 255)

Occurrence. Vancouver Island (*1*), (*2*), (*3*), dredged in 36, 130, 137 metres, respectively.

Remarks. This species is probably synonymous with *A. japonica* McIntosh (47, p. 431). It occurs on the east coast of Vancouver Island (authors' unpublished record).

Amphicteis mucronata Moore (64, p. 203; 8, p. 310)

Occurrence. Vancouver Island (*3*), dredged in 137 to 180 metres. Tassoo Harbour, Queen Charlotte Islands, dredged.

Sabellides octocirrata Sars (25, p. 232)

Occurrence. Nash Harbour, Nunivak Island, and Spiriden Bay. Kodiak Island, Alaska, dredged in 8 to 12 fathoms.

Remarks. This species is found in fine mud tubes resembling those of *Myriochele heeri* Malmgren. It occurs on the east coast of Vancouver Island (authors' unpublished record).

Amage anops (Johnson) (15, p. 52)

Sabellides anops (42, p. 424)

Occurrence. Vancouver Island (*3*), dredged in 75 to 90 metres; Humpback Bay, Alaska, dredged in 15 fathoms.

Melinna cristata (Sars) (25, p. 237; 8, p. 311)

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Lysippe labiata Malmgren (48, p. 367)

Occurrence. Cleveland Passage, Alaska.

Samytha sexcirrata (Sars) (48, p. 370; 20, p. 23B; 64, p. 214)

Occurrence. Cleveland Passage, Alaska.

Remarks. The single specimen agrees closely with the descriptions except that there are 20 abdominal segments, which is more than is recorded for the species. McIntosh (46, p. 78) gives "12 to 18". The number of these segments is somewhat variable in the genus.

Family TEREBELLIDAE

Amphitrite cirrata (O. F. Müller) (25, p. 251; 20, p. 22B; 15, p. 52)

Occurrence. McClinton Bay, Masset Inlet (collector, Dr. A. L. Pritchard); Pacofi Bay, Skidegate Narrows. All Queen Charlotte Islands, littoral.

Neoamphitrite robusta (Johnson) (36, p. 17; 75, p. 155)

Amphitrite robusta (42, p. 425), *Terebella robusta* (11, p. 773)

Occurrence. Vancouver Island (*2*), dredged in 555 metres, (*3*), dredged in 170 to 180 metres and littoral, (*4*), littoral. Louscoone Bay, Queen Charlotte Islands, littoral. Ketchikan, Alaska, littoral.

Remarks. The example collected at the greatest depth (555 metres) has a hard tube constructed of coarse sand, quite unlike the muddy tube in which the species is found between tide-marks, but no difference can be observed in the technical characters of the animal.

Terebella ehrenbergi Grube (26, p. 226; 27, p. 80)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. The majority of records of this species are from warmer seas. It has not been recorded previously from the west coast of North America. It appears to be fairly common in Japan and has been found recently associated with introduced Japanese oysters at Ladysmith Harbour on the east coast of Vancouver Island. It may well have reached the coast via Japan.

Pista cristata (Müller) (25, p. 266; 60, p. 351; 15, p. 53)

Occurrence. Vancouver Island (*3*), dredged in 137 metres; Spiriden Bay, Kodiak Island, Alaska.

Pista fratrella Chamberlin (19, p. 18; 8, p. 309; 15, p. 53)

Occurrence. Vancouver Island (*2*), dredged in 130 and 172 metres, (*3*), dredged in 75 to 90 metres; off Cape Cook, west coast of Vancouver Island in a deep net haul (collector, Dr. H. C. Williamson).

Pista pacifica sp. n.

Pista elongata (11, p. 773 (non Moore))

Occurrence. Vancouver Island (*4*), littoral.

Remarks. Four anterior portions of this species are in the collection together with a number of empty tubes. The characters are exactly those of the forms from Elkhorn Slough, California, described by the writers under the name *P. elongata* Moore. The writers pointed out wherein they differed from this latter species, but regarded the differences as coming within the limits of variation. Examination of the present specimens convinces them that this is not so. The outstanding differences are the presence of a definite tongue-shaped wing on IV (Fig. 1), which is entirely absent in *P. elongata*, and the form of the long-stemmed thoracic uncinus (Fig. 2).

The tubes of the two species are quite different. That of *P. elongata* has been described by one of the writers (8, p. 309). That of the present species has a heavy coating of mud and sand and terminates in a hood bent at an angle to the tube and ornamented round the mouth with long pendant processes (Fig. 3).

P. macrolobata Hessle has lobes on the anterior segments very similar to those of this species, but differs in having only two pairs of branchiae and in the form of the long-stemmed thoracic uncinus.

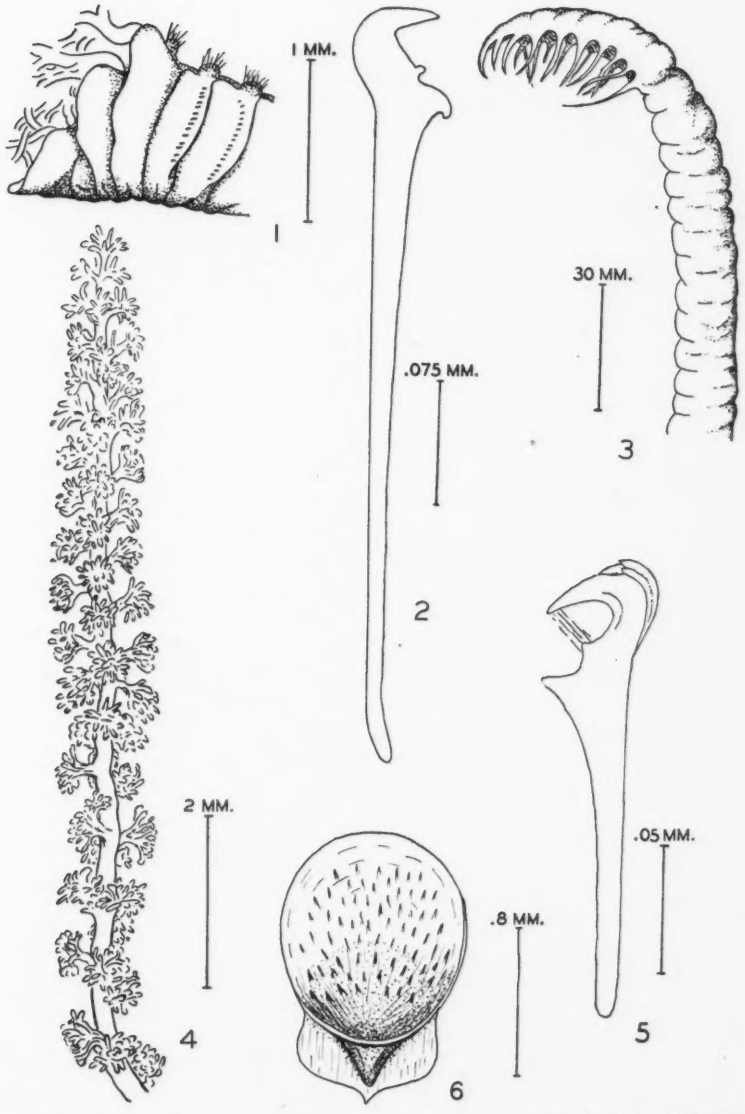

FIG. 1. *Pista pacifica sp. n. Anterior end.* FIG. 2. *Pista pacifica sp. n. Thoracic uncinus.* FIG. 3. *Pista pacifica sp. n. Tube.* FIG. 4. *Pista moorei sp. n. Branchia.* FIG. 5. *Pista moorei sp. n. Thoracic uncinus.* FIG. 6. *Spirorbis medius Pixell. Opercular plate newly regenerated.*

Pista moorei sp. n.

Pista sp.? (64, p. 197)

Occurrence. Vancouver Island (*1*), dredged in 36 metres.

Remarks. The single example is almost certainly identical with the species described from southern California by Moore, but left unnamed by him because his material was imperfect. The writers have examined other specimens from the east coast of Vancouver Island and from Monterey Bay, California, and base this opinion on a consideration of all the material. The gills and the wings on the anterior segments correspond with Moore's description and the former are unlike those of any other species of *Pista* known to the writers. When fully expanded they are long and trailing and have whorls of branchlets at intervals throughout their length (Fig. 4). The wings on I and II are generally as Moore describes, but the development of those on III varies considerably in different specimens. They are always large and extend from the ventral side to above the dorsum, but the tongue-like extension may rise only a little above the dorsum or so much above it as to reach beyond the prostomium when directed forward, as Moore describes. The wings on IV correspond with Moore's description, but are not placed as far ventrally as those on II in the writers' specimens.

An uncinus from the first thoracic torus is shown in Fig. 5.

Thelepus crispus Johnson (42, p. 428; 61, p. 275)

Occurrence. Vancouver Island (*2*), and (*4*), littoral. Pacofi Bay and Skidegate Narrows, Queen Charlotte Islands, littoral.

Remarks. This species is similar to *T. setosus* (Quatrefages) and the writers have (14) expressed the opinion that they were indistinguishable. The material in this collection shows this opinion to have been erroneous. The two species are most readily differentiated by the arrangement of the uncini. Excepting on the first few somites these form flattened rings in *T. crispus*, whilst they remain single lines throughout the body in *T. setosus*. Other points of difference are:—

1. The shape of the uncinigerous pinnules, which are wart-like and not very prominent in *T. crispus*, square and projecting in *T. setosus*.

2. The extension of the notopods, which persist until near the posterior extremity in *T. crispus*, but cease many segments short of it in *T. setosus*.

3. The definition of the ventral plates. These are fairly well defined in *T. setosus*, but are so much furrowed and folded in *T. crispus* as to be almost indistinguishable. The species has not been recorded previously north of California, but it has been taken at Port Townsend, Washington (collector, Mr. E. F. Ricketts; authors' identification).

Polycirrus kerguelensis (McIntosh) (53, p. 194)

Ereutho kerguelensis (43, p. 474)

Occurrence. Vancouver Island (*2*), dredged in 58 metres.

Remarks. A single complete example agrees exactly with the form described by McIntosh as *Ereutho kerguelensis* except in that it has 12 thoracic notopodia, whilst McIntosh gives 13. Monro (55, p. 184) finds that the number may vary from 11 to 15.

This form is to be distinguished from that described by McIntosh (43, p. 475) as *Polycirrus kerguelensis*, which has a different form of uncinus and agrees with *Ereutho antarctica* Willey (16, p. 95).

Polycirrus caliendrum Claparède (25, p. 281)

Polycirrus perplexus (64, p. 198; 8, p. 310)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. There seems to be nothing to differentiate this form from Moore's *P. perplexus*. The form of the uncinus is very variable and no valid distinction can be based upon it. Fauvel points out that *P. caliendrum* is very similar to, if not identical with *P. nervosus* Marenzeller, which is recorded from Japan (27, p. 83).

Terrebellides stroemi Sars (25, p. 291; 60, p. 352; 8, p. 310; 20, p. 23B)

Occurrence. Vancouver Island (*3*), dredged in 89 to 180 metres. Spiriden Bay, Kodiak Island, Alaska. Nash Harbour, Nunivak Island, Alaska, dredged in 8 to 10 fathoms.

Scionella estevanica sp. n.

Occurrence. Vancouver Island (*3*), dredged in 137 metres.

Description. Two specimens, of which the posterior region is incomplete, occur in the collection. The body is long and not more than 1 mm. wide, considerably more slender than *Scionella japonica* Moore (56, p. 473), which appears to be the only other known representative of the genus. It resembles the latter species closely in all other morphological characters except the following:

1. There is a long low ridge on the dorsum of IV instead of the shorter high fold of *S. japonica*.
2. The lateral lobes of the first four somites do not rise successively as abruptly as in *S. japonica*, so that those of IV do not attain as dorsal a position.
3. The single pair of branchiae is attached to the anterior edge of II instead of to the fold on IV as in *S. japonica*.
4. The branchiae are bottle-brush shaped and have about 20 densely packed whorls.

The tubes are thin and membranous and are beset with sand grains. They fit the animal very tightly.

Scionella japonica has been recorded from Friday Harbour, Washington (76) and occurs on the east coast of Vancouver Island (authors' unpublished record).

Neoleprea spiralis (Johnson) (36, p. 17)

Amphitrite spiralis (42, p. 426; 8, p. 308)

Occurrence. Vancouver Island (*2*), littoral.

Artacama conifera Moore (58, p. 853; 64, p. 191)

Occurrence. Nootka Sound, west coast of Vancouver Island (collector, J. P. Tully).

Remarks. This species has been recorded from Friday Harbour, Washington (76) and has also been taken off the east coast of Vancouver Island (authors' unpublished record).

Family SABELLIDAE

Demonax leucaspis Kinberg (54, p. 1075 (with synonymy); 15, p. 54)

Occurrence. On an old pile below low-water-mark at Ucluelet, west coast Vancouver Island (collector, Dr. J. L. Hart).

Eudistylia vancouveri (Kinberg) (36, p. 22)

Eudistylia gigantea (17, p. 210; 9, p. 70; 10, p. 315)

Occurrence. Vancouver Island (*4*), littoral.

Eudistylia polymorpha (Johnson) (36, p. 23)

Bispira polymorpha (42, p. 428; 9, p. 70)

Occurrence. Vancouver Island (*2*), dredged in about 555 metres.

Distylia rugosa Moore (57, p. 499; 61, p. 289; 9, p. 69)

Occurrence. Vancouver Island (*2*), littoral.

Potamilla torelli Malmgren (25, p. 310)

Occurrence. Vancouver Island (*3*), littoral.

Remarks. This species is distinguished by the absence of branchial eyes. Two specimens, apparently mature, occur in the collection and correspond closely with Fauvel's description except that no pigment remains. Several younger forms, differing by having fewer branchial filaments (sometimes as few as three on each side) and very pointed ends to the collar, also occur. All were found in fragile tubes embedded in the walls of the tubes of *Idanthyrsus armatus* Kinberg.

Pseudopotamilla reniformis (Leuckhart) (25, p. 309)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. This species, widely distributed in Arctic seas and elsewhere, has been recorded from Alaska (60, p. 359) and from Vancouver Island (9, p. 70; 10, p. 315). Hartman (36, p. 27) expresses doubt as to the validity of these records in view of the possible confusion of this species with *P. intermedia* Moore. The writers are inclined to regard the latter as a synonym, the only significant difference being that whilst *P. intermedia* is said to have never more than two rather inconspicuous eyes, and sometimes only one or none, on a radiole, *P. reniformis*, as described by Fauvel, may have as many as eight large conspicuous ones. However, other authors have failed to observe more than two eyes. This latter condition holds in the specimens in the present collection. They have eight thoracic setigers, resembling in this and other respects the specimens previously described from Vancouver Island. Fauvel gives 9 to 12, Johansson (40, p. 143) seven to nine. It is possible that the species referred to by Hartman as *P. reniformis* (Montagu) is a different form since she describes it as a "warm water species".

Branchiomma burrardum Berkeley (9, p. 71; 15, p. 55)

Occurrence. Vancouver Island (*3*), dredged in 170 to 180 metres.

Schizobranchia nobilis Bush (17, p. 207; 9, p. 71)

Occurrence. Vancouver Island (*3*), littoral. Pavlov Bay, Alaska.

Schizobranchia insignis Bush (17, p. 206; 9, p. 70)

Occurrence. Vancouver Island (*4*), littoral. Pavlov Bay and Cleveland Passage, Alaska.

Remarks. The writers are very doubtful whether this and the preceding species should be separated. The characters on which differentiation has been based are very variable and the two forms are found together.

Fabricia sabella (Ehrenberg) (25, p. 325; 9, p. 73)

Occurrence. Vancouver Island (*4*), littoral.

Chone gracilis Moore (59, p. 257; 10, p. 315)

Occurrence. Pavlov Bay, Alaska.

Chone infundibuliformis Kröyer (25, p. 334; 15, p. 55)

Occurrence. Vancouver Island (*4*), littoral; Nash Harbour, Nunivak Island, Alaska, dredged in 8 to 10 fathoms; Pavlov Bay, Alaska.

Euchone analis (Kröyer) (20, p. 27B; 9, p. 71)

Occurrence. Cleveland Passage, Alaska.

Family SERPULIDAE

Serpula vermicularis Linné (25, p. 351; 9, p. 73; 47, p. 353)

Occurrence. Vancouver Island (*2*), (*3*), (*4*), all littoral.

Salmacina dysteri (Huxley) var. *tribranchiata* (Moore) (54, p. 1090; 15, p. 56)

Occurrence. Vancouver Island (*4*), littoral.

Crucigera irregularis Bush (66, p. 786; 9, p. 73)

Occurrence. Vancouver Island (*2*), littoral and dredged in 172 metres, (*3*), dredged in 113 and 155 metres. Humpback Bay, Alaska, dredged in about 15 fathoms.

Crucigera zygophera (Johnson) (66, p. 786; 9, p. 73)

Serpula zygophera (42, p. 433)

Occurrence. Vancouver Island (*2*), dredged in 48 metres.

Chitinopoma groenlandica Mörch (66, p. 790; 9, p. 74)

Occurrence. Vancouver Island (*2*), dredged in 64, 70, and 130 metres.

Spirorbis racemosus Pixell (66, p. 799)

Occurrence. Vancouver Island (*1*), littoral.

Remarks. This species does not appear to have been recorded since it was originally described from the east coast of Vancouver Island. It is noteworthy that the present specimens were collected between tide-marks whereas the original ones were dredged in 15 to 25 fathoms.

Spirorbis medius Pixell (66, p. 800; 68, p. 149; 25, p. 398)

Occurrence. Vancouver Island (*4*), littoral.

Remarks. A number of representatives of this species in the collection have no notch in the serrated edge of the collar setae. Others have only one or two setae notched, the remainder with unbroken edge. This is in agreement with Southern's findings in Irish specimens. The calcareous plate of the operculum can be regenerated. Pixell's figure represents a worn plate. The newly regenerated plate is densely covered with sharp conical transparent spines and the wings are wider than they are when it is worn (Fig. 6).

Spirorbis ambilateralis Pixell (66, p. 798; 9, p. 74)

Occurrence. Vancouver Island (*2*), littoral.

Spirorbis spirillum (Linné) (25, p. 392; 66, p. 796; 9, p. 74)

Occurrence. Vancouver Island (*3*), littoral.

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THE EFFECT OF ASPHYXIATION UNDER VARIOUS TENSIONS OF CARBON DIOXIDE ON THE SWIM BLADDER GASES OF SOME FRESHWATER FISH[1]

By Virginia Safford Black[2]

Abstract

Freshwater physostomous fish, possessing open swim bladders (dace, sucker, chub, bullhead, trout), and physoclistous fish, having closed swim bladders (perch, bass, pumpkin-seed, stickleback), were sealed in bottles of water saturated with air and containing various amounts of dissolved carbon dioxide. After asphyxiation, an analysis of the swim bladder gases showed that the swim bladder had gained some carbon dioxide and lost some oxygen. Fish with closed swim bladders possessing *retia mirabilia* and ovals, vascular networks that facilitate gas exchange, show a markedly greater exchange of gases during the experiments than fish with open swim bladders which have no intensely vascular regions on the swim bladder wall. The oxygen that leaves the swim bladder during asphyxiation is probably of no significant value to the fish for respiration.

Introduction

The main function of the swim bladder of fish is that of adjusting the buoyancy of the fish at various depths and pressures. Further functions attributed to it are respiration (16, 20, 22), hearing (3, 18), sound production (25), pressure sensitivity (2, 4), and co-ordination of movement (26). The present paper deals with the exchange of gases at the swim bladder, a process involved in buoyancy adjustments and in respiration.

The experiments show the response of two types of swim bladder to changes in the gas content of the water. One of these types is the open or physostomous swim bladder. It is connected to the anterior gut by an open duct, the *ductus pneumaticus*. Regulation to hydrostatic pressure may be made by the passage of gas to or from the swim bladder by way of the duct. The second type is the closed or physoclistous swim bladder whose duct is closed or completely atrophied at a very early stage in the life of the fish. All gaseous regulation to increased external pressure in these fish must be made by secreting gas into the bladder through the combined effort of the *rete mirabile* and gas gland of the swim bladder. Gas is absorbed again into the blood at the oval, a section of the swim bladder wall whose vascular supply is made available by nervous control when there is a decrease in hydrostatic pressure (17, 19). Some physostomes also have *retia mirabilia*, but none of the physostomous species represented in this paper are in that group.

The proportion of carbon dioxide, oxygen, and nitrogen in the swim bladders of fish living in water equilibrated with air appears to be charac-

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[2] *Assistant, Department of Zoology, University of Toronto, Toronto, Ont.*

TABLE I

GAS CONTENT OF THE SWIM BLADDERS OF CONTROL FISH

	Carbon dioxide			Oxygen		
	Average %	σM	Number of fish	Average %	σM	Number of fish
Physoclists:						
Bass	1.4	±0.50	5	26.0	±0.64	5
Pumpkin-seed	0.9	±0.17	10	21.2	±1.2	10
Stickleback	1.2	±0.30	5	19.6	±0.87	5
Perch	1.3	±0.27	12	19.4	±0.71	12
Physostomes:						
Dace	4.9	±0.71	9	12.1	±1.4	9
Sucker	1.9	±0.26	5	11.1	±0.49	5
Bullhead	0.6	±0.08	12	7.5	±0.64	12
Chub	2.4	±0.55	10	6.4	±1.2	10
Trout	0.8	±0.17	13	6.0	±0.71	13

TABLE II

A. COMPARATIVE DATA ON CARBON DIOXIDE AND OXYGEN PERCENTAGES FOUND IN THE SWIM BLADDERS OF CONTROL FISH LIVING IN WATER EQUILIBRATED WITH AIR

Species	No. of fish	CO_2, %	σM	O_2, %	σM	Place of collection	Reference
Perca flavescens	28	0.63	±0.03	19.9	±0.88	Wisconsin	(8)
	12	1.3	±0.27	19.4	±0.71	Ontario	Table I
Micropterus dolomieu	3	2.5	±0.84	24.6	±5.8	Wisconsin	(8)
	5	1.4	±0.50	26.0	±0.64	Ontario	Table I
Lepomis gibbosus	9	0.65	±0.07	21.9	±0.61	Wisconsin	(8)
	10	0.9	±0.17	21.2	±1.2	Ontario	Table I
Catostomus commersonnii	1	4.3	—	5.4	—	Wisconsin	(8)
	21	4.6	±0.36	13.6	±0.27	Pennsylvania	(24)
	5	1.9	±0.26	11.1	±0.49	Ontario	Table I

B. COMPARATIVE DATA FOR PHYSOCLISTS AND PHYSOSTOMES SHOWING A SIGNIFICANT DIFFERENCE IN CARBON DIOXIDE AND OXYGEN PERCENTAGES

	No. of species	CO_2, %	σM	O_2, %	σM		Reference
Physoclists	20	1.0	±0.11	22.2	±2.4		(8, 12, 23) and Table I
Physostomes	15	2.8	±0.45	10.4	±2.6		(8, 12, 24) and Table I
Δ		1.8	±0.46	11.8	±3.5		

teristic of the species (Table IIA), but subject to variation during gas secretion and resorption in response to changes in hydrostatic pressure (8, 11). Since the swim bladders of the physostomes are exposed chiefly to gases in the gut one finds in most species a higher carbon dioxide and lower oxygen percentage (Table IIB) than is present in the water. The physoclists, as shown in Tables I and IIB, usually have a fairly low carbon dioxide percentage and an oxygen percentage close to or higher than that of water saturated with air (21% oxygen). The relation between the gases of the water and swim bladder is probably more marked in the physoclists because gas exchange takes place by way of the blood both at the gills and at the *rete mirabile* and oval of the swim bladder.

It has been shown by experiment that (*a*) when the carbon dioxide in the water is increased the carbon dioxide content of the swim bladder also rises (8, 21), (*b*) when the oxygen in the water is decreased the percentage of oxygen in the swim bladder also decreases (8). In the present paper (*a*) and (*b*) are combined since the carbon dioxide in the water is increased to various tensions and the fish lowers the oxygen tension to the asphyxial point in each.

The asphyxial oxygen tension at the gills is influenced by the tension of carbon dioxide in the water so that when the carbon dioxide is increased beyond a certain tension, characteristic for each species, the oxygen tension at asphyxiation is also higher (6, 7). The changes in the swim bladder gases during asphyxiation in this manner have been followed in some physoclistous marine fish with the following results (23): (*a*) carbon dioxide enters the swim bladder reaching a pressure comparable to the carbon dioxide tension of the water until the latter is so high that it can be tolerated by the fish only a short time, thus decreasing the time for equilibration before death, (*b*) oxygen disappears from the swim bladder during asphyxiation and in a few cases was entirely lacking at the end of the experiment, (*c*) the extent of disappearance of oxygen appeared to depend on the carbon dioxide and oxygen tension of the water, reflecting the condition at the gills.

Experiments carried out in the summer of 1940 on some of the freshwater fish in Algonquin Park, Ont., are reported in this paper and the results compared with those of similar work on marine fish (23).

Materials and Methods

The four species of physoclists used were the northern smallmouth bass, *Micropterus dolomieu* Lacépède; the pumpkin-seed, *Lepomis gibbosus* (Linnaeus); the yellow perch, *Perca flavescens* (Mitchill); the brook stickleback, *Eucalia inconstans* (Kirtland). The physostomes are represented by the finescale dace, *Pfrille neogaea* (Cope); the common white sucker, *Catostomus commersonnii* (Lacépède); the northern creek chub, *Semotilus atromaculatus* (Mitchill); the northern brown bullhead, *Ameiurus nebulosus* (LeSueur); the common brook trout, *Salvelinus fontinalis* (Mitchill). The nomenclature is taken from Hubbs and Lagler (10).

All the species except the sucker and trout were hooked, seined, or trapped in the surface waters of Lake Opeongo or small neighbouring lakes. The suckers were trapped at a depth of about 30 ft. The trout had been raised by the Department of Game and Fisheries of Ontario.

From the point of view of their resistance to asphyxiation the previous history of the fish apparently made little difference, provided that experiments were done at very nearly the temperature from which the fish had come. The swim bladder gases, on the other hand, are influenced by the hydrostatic pressure on the fish. Hence it was desirable to keep the fish caught in deep water (some bass and perch, all suckers) in surface water for a day before using them for experiment. Complete adjustment to pressure could be made in this time (1), so that no gas exchange resulting from change in external pressure would occur during the experiments. Previous to the experiments all the fish were living in lake water containing at all times less than 10 mm. carbon dioxide and over 120 mm. oxygen.

The procedure and analytical methods were identical with those used for marine fish (23), i.e. each fish was asphyxiated in a sealed bottle of water containing carbon dioxide at tensions from 4 to 300 mm. and oxygen at tensions from 100 to 250 mm. depending on the individual experiment (7). After asphyxiation the oxygen in the water was determined by the Winkler method, the carbon dioxide by equilibration of the water with a small bubble of air which was then analysed for carbon dioxide by a modification of Krogh's micromethod (14). The latter method was also used for determining the percentage of carbon dioxide and oxygen in the swim bladder gas. The apparatus used in this work was accurate to 0.5 of 1%. The temperature of the experiments ranged from 17° to 22° C. No effect of temperature was apparent within this range. The swim bladder gases of control fish, caught and kept in the same manner as the fish used for the experiments, were also analysed.

The duration of the experiments varied from one to four hours for all species except two. These were the dace, which sometimes continued respiratory movements as long as five hours, and the bullhead which lived up to seven hours in a sealed bottle. In general the experiments at low carbon dioxide tensions lasted longer than those at high carbon dioxide tensions presumably because the ability of the fish to use oxygen decreased as the carbon dioxide in the water was increased.

The pressures of the swim bladder gases, recorded for comparison with water tensions, have been calculated for Table III and Fig. 1 on the assumption that the gases in the swim bladder were at atmospheric pressure. This was not so in the cyprinoid fishes (chub and dace) nor in the sucker where the pressure within the swim bladder was often sufficient to displace the plunger of the syringe (cf. Reference 3). However, all pressures have been calculated from percentages as follows: the percentage of gas (10% carbon dioxide, for example) was multiplied by the barometric pressure (in mm. mercury) at 1300 ft. less the water vapour pressure (in mm. mercury) at the temperature

of the experiment (725−14.5 at 17.0° C.), giving 71.0 mm. carbon dioxide. The laboratory is located at an altitude of 1300 ft.

The curves in Fig. 1 were drawn by inspection of the plotted data. The standard deviation of the mean (σM) is recorded for the data presented in Tables I, II, and IV.

TABLE III

THE RANGE OF PRESSURES OF CARBON DIOXIDE AND OXYGEN FOUND IN THE WATER AND SWIM BLADDER AFTER ASPHYXIATION. A SUMMARY OF ALL THE EXPERIMENTS

	No. of fish	Gases in water		Gases in swim bladder			
		CO_2, mm.	O_2, mm.	CO_2		O_2	
				mm.*	%	mm.*	%
Physoclists:							
Perch	25	5– 88	5–109	7– 75	0.9–10.7	24–131	3.4–18.6
Bass	17	6– 95	12–186	7– 78	0.8–11.1	40–195	3.8–28.4
Pumpkin-seed	20	4–119	5–180	11– 66	1.6– 9.4	23–121	3.4–17.1
Stickleback	20	4–195	13–175	17–202	2.4–28.6	18– 65	2.6– 9.2
Physostomes:							
Sucker	16	7– 99	6–168	14– 37	2.0– 5.2	48– 99	6.8–14.1
Trout	29	5–137	16–203	3– 97	0.4–13.9	3–112	0.4–16.0
Dace	25	6–200	4–172	37–125	5.2–17.8	6–169	0.8–24.0
Chub	25	4–202	5–112	21– 78	3.0–11.1	20–151	2.9–21.6
Bullhead	20	7–300	1–119	5– 82	0.7–11.8	13– 92	1.8–13.1

* *Pressures of swim bladder gases calculated by assuming atmospheric pressure in the bladder.*

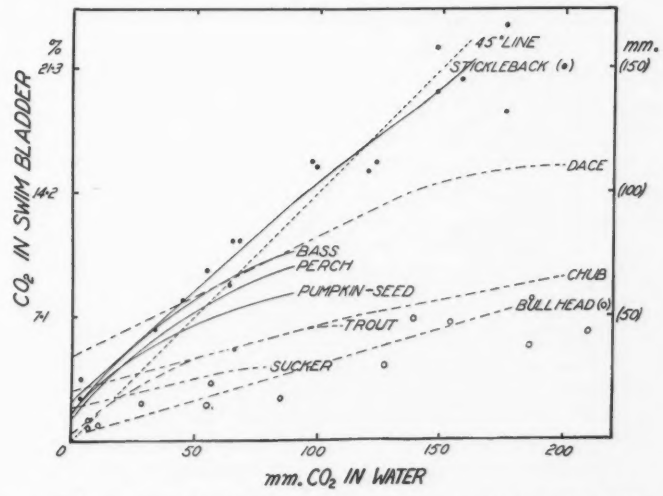

FIG. 1. *Relation of carbon dioxide in water to carbon dioxide in swim bladder after asphyxiation.* ——— = *physoclists;* – – – – = *physostomes.*

Results

The specific nature of the proportion of gases in the swim bladder is brought out in Table II. Here the carbon dioxide and oxygen percentages for control fish (Table I) are compared with the percentages found by Hall (8) for the same species.

The data for all the experiments have been summarized in Table III to show the range of carbon dioxide tensions used for each species, the resulting asphyxial oxygen tensions, and the range of carbon dioxide and oxygen pressures in the swim bladder, all determined after the asphyxiation of the fish. The number of fish used, i.e., the number of single experiments, is indicated. During the experiments the carbon dioxide in the water decreased slightly; the carbon dioxide tension after asphyxiation was rarely less than 4/5 of the initial tension. The oxygen in the water decreased to the asphyxial oxygen level which varied with each species and carbon dioxide tension. The carbon dioxide in the swim bladder increased, the oxygen appeared to decrease or show little change. The table serves chiefly to give a general comparison of the gases in the water with those in the swim bladder and to show the tensions of carbon dioxide that each species can tolerate. The physoclists and physostomes are listed in order of increasing ability to tolerate carbon dioxide.

The relation between the carbon dioxide in the water and in the swim bladder at the time of asphyxiation is given in Fig. 1. The data are plotted for the stickleback and bullhead up to 210 mm. carbon dioxide. The remaining points are beyond the scope of this figure but are included in the tables. It is clear that carbon dioxide enters the swim bladder in all these species but it is also apparent that the physoclists are in closer equilibrium with the water than are the physostomes. The latter show a slow reaction to the carbon dioxide in the water and rarely attain a carbon dioxide pressure in the swim bladder equivalent to that of the water unless it was present initially. Though the line for the dace seems high because of the high initial carbon dioxide in the bladder the actual slope is more gradual than the slope of the physoclists. The marine and freshwater physoclists, however, keep in the neighbourhood of the 45° line until the carbon dioxide in the water becomes so great that asphyxiation occurs before the equilibration takes place. It may be concluded, therefore, that carbon dioxide enters the swim bladder of physoclists more readily than the swim bladder of these physostomes.

The ability of freshwater and marine fish to use the oxygen in the swim bladder during asphyxiation is compared in Table IV. The results of experiments at all carbon dioxide tensions are averaged for each species (Asphyxiated). These averages are compared with the controls (Control), and the actual (ΔO_2) and percentage differences in oxygen are noted. After asphyxiation there is a significant decrease in the oxygen in the swim bladder of all the physoclists except the scup. The physostomes, on the other hand, show no significant difference in the proportion of oxygen in the swim bladder

TABLE IV

CHANGE IN THE OXYGEN CONTENT OF THE SWIM BLADDER IN ASPHYXIATION. AN AVERAGE OF ALL EXPERIMENTS COMPARED WITH THE CONTROLS

	Control			Asphyxiated			ΔO_2	Decrease in O_2, %
	O_2, %	σM	No. fish	O_2, %	σM	No. fish		
Marine fish								
Physoclists:								
Scup	9.4	±1.6	4	7.0	±0.94	8	− 2.4±1.8	25
Cunner	25.8	±1.5	4	13.2	±1.7	19	− 12.6±2.2	49
Killifish	15.6	±2.3	16	7.3	±0.51	22	− 8.3±2.4	53
Tautog	51.3	±3.4	4	14.6	±4.6	11	− 36.7±5.7	72
Toadfish	55.0	±3.2	32	15.0	±4.2	16	− 40.0±5.1	73
Sea robin	22.8	±1.7	6	3.6	±0.91	8	− 19.2±1.9	84
Freshwater fish								
Physoclists:								
Bass	26.0	±0.64	5	17.6	±1.4	17	− 8.4±1.4	32
Perch	19.4	±0.71	12	10.7	±0.88	25	− 8.7±1.1	45
Pumpkin-seed	21.2	±1.2	10	9.7	±1.1	20	−11.5±1.6	54
Stickleback	19.6	±0.87	5	5.6	±0.54	20	−14.0±1.0	72
Physostomes:								
Chub	6.4	±1.2	10	8.9	±0.95	25	+ 2.5±1.6	—
Trout	6.0	±0.71	13	6.4	±0.71	29	+ 0.4±1.0	—
Sucker	11.1	±0.49	5	9.7	±0.53	16	− 1.4±0.72	13
Dace	12.1	±1.4	9	9.7	±1.3	25	− 2.4±2.0	20
Bullhead	7.5	±0.64	12	6.0	±0.58	20	− 1.5±0.87	20

of control and asphyxiated fish, and in no case is the percentage decrease in oxygen as high as the lowest value found in the physoclists. It appears that little, if any, oxygen in the swim bladder is available to the physostomes whereas the physoclists lose from 25 to 84% of their swim bladder oxygen during asphyxiation.

The above paragraphs present the general effect of asphyxiation on the swim bladder gases when carbon dioxide is present in the water. The course of the individual experiments for the physostomes, with respect to carbon dioxide, is shown in Fig. 1. No trend is consistently exhibited in the swim

Carbon dioxide in water	Carbon dioxide in swim bladder		Oxygen in swim bladder	
	Freshwater fish	Marine fish	Freshwater fish	Marine fish
None added	Low	Low	High	Low
Intermediate	Increasing	Increasing	Low	Increasing
High	High or variable	High or variable	High (i.e. near normal O_2 percentage)	High or variable

bladder oxygen with an increase in the carbon dioxide in the water or swim bladder. The gases in the physoclist swim bladder, however, assume the following pattern as the carbon dioxide in the water is increased.

Hence it is apparent that the carbon dioxide in the swim bladder is directly controlled by the carbon dioxide in the water, but it is difficult to see what factor is responsible for the changes in oxygen. The difference between the proportion of oxygen in the swim bladder of freshwater and marine fish at low carbon dioxide tensions is significant, and shows that a factor other than simple diffusion determines the oxygen in the swim bladder. Since freshwater fish have a higher alkaline reserve than marine fish (5), small amounts of carbon dioxide would have less effect on the internal environment; the situation for freshwater fish at intermediate carbon dioxide tensions might therefore actually be equivalent to that of marine fish at low carbon dioxide tensions.

The stickleback (average weight, 0.6 gm.; volume of gas, about 0.05 cc.) differs from the other freshwater physoclists in the consistently low oxygen content of its swim bladder after asphyxiation no matter how high the carbon dioxide or oxygen in the water. This high utilization of swim bladder oxygen in asphyxiation may be due to respiration of the cells of the swim bladder wall or neighbouring tissues. Changes brought about in this way might show up in the stickleback because of the very small volume of gas. However, a similar situation occurs in the swim bladder of the marine fish, the sea robin, which is a good-sized fish with an exceptionally well developed *rete mirabile* (average weight of sea robin, 120 gm.; volume of gas, 10 to 15 cc.).

Discussion

The data show that during asphyxiation a change occurs in the proportion of gases in the swim bladder toward equilibration with the gas content of the water. The change is more marked in the physoclistous fish than in the physostomous species. Two questions arise from these facts: (*a*) the method of passage of gases to and from the swim bladder, (*b*) the reason for the difference in the quantity of exchange in the two types.

Gas can enter or leave the swim bladder either by passing through the swim bladder wall or by diffusing to and from the blood. (Passage of gas through the duct of the physostomes was not a factor in the experiments.) There are conflicting ideas about the permeability of the swim bladder wall to gases and it is possible that permeability varies with the species for there is great morphological variation. In any case, even in the swim bladders that appear permeable, carbon dioxide is the gas that most readily passes the wall, oxygen taking weeks to diffuse out (11, 15). The data for the physostomes indicate little change in the percentages of the gases during asphyxiation. Therefore loss or gain through the swim bladder wall cannot be large during the time of the experiments. Since the data for the physoclists show a very appreciable decrease in oxygen within one to four hours. the greater proportion of gas exchange must be by way of the blood.

The physostome swim bladder is supplied with blood vessels that are distributed over the swim bladder wall so that it is by no means lacking in blood supply. However, these species do not possess the specialized vascular networks found in the physoclists. Precisely how and when the networks function in the course of these experiments is a matter of conjecture. Normally the oval absorbs gas from the swim bladder and it is probable that carbon dioxide enters at both the oval and *rete mirabile* in the experiments.

Other factors that undoubtedly affect the gas exchange at high carbon dioxide tensions are: (*a*) higher oxygen tension in the water at asphyxiation, (*b*) less time for equilibration because of shorter duration of the experiments, (*c*) the physiological effect of carbon dioxide in the swim bladder on the combination and dissociation of oxygen and blood, (*d*) decrease in heart-beat rate (9), resulting in slower circulation.

It is apparent from Table IV that the average percentage of oxygen in the swim bladder of asphyxiated fish is similar for both physoclists and physostomes. It might be argued that the physoclists absorb a higher percentage of oxygen only because they have more to begin with, whereas the physostomes reach the same "minimum" by absorbing very little oxygen. The following considerations indicate an *actual* greater absorption of oxygen by physoclists than by physostomes. (*a*) The experimental physostomes show as much variation as the controls, but the oxygen in the swim bladders of the physoclistous fish is influenced by the experimental conditions. (*b*) The fact that carbon dioxide does not readily enter the physostome swim bladder might indicate that oxygen does not readily disappear. (*c*) Khalil (13) found that a carp (physostome) whose swim bladder oxygen had been artificially enriched to 26.3% absorbed 3% in five hours when in oxygen-poor water. The oxygen content of the control bass averages 26% (Table IV), the asphyxiated bass averaging 17.6% in experiments lasting less than five hours. In a comparable situation then the physostome absorbed only 3%, whereas the physoclist absorbed 8% of its swim bladder oxygen.

The disappearance of some of the oxygen from the swim bladder of the physoclists brings up the question of the use of the swim bladder as a source of oxygen in respiratory stress. The function of the swim bladder as an accessory organ of respiration has been clearly shown by Potter (20) for some of the bony ganoid fishes with open swim bladders, such as the gar-pike and bowfin, which come to the surface and gulp air into the bladder. The structure of their swim bladders is more complex and the circulation adapted for respiration. The teleost physostome, *Umbra krameri*, a European freshwater fish, also uses its swim bladder as an accessory organ of respiration but its swim bladder is provided with gas gland, *rete mirabile*, and vascular epithelium (22). The swim bladders of the teleost physostomes of this paper, however, do not show any adaptation for a respiratory function. In the physoclists the facility of gas exchange by means of the blood is a necessity for regulation to external pressure since there is no other way for gas to enter or depart. It seems plausible, therefore, that oxygenated blood leaving the

swim bladder might serve to benefit other tissues. However, the availability of the oxygen to other tissues for respiration appears unlikely since the blood leaving the swim bladder goes either directly, or by way of the liver, to the heart and thence to the gills for oxygenation. En route it is joined by vessels of deoxygenated blood from the rest of the body so that its effectiveness as an oxygen carrier is shortlived and inefficient. Experiments and calculations by Hall (8) show that only about 1 cc. of oxygen disappears from the swim bladder of perch kept in water of low oxygen content for five hours, the oxygen consumption of the fish being about 6 cc. per hr. Likewise extensive work by Khalil (13) on the significance of the swim bladder in respiration indicates that the Europen tench and carp (physostomes) kept in oxygen-poor water for seven or eight hours, absorb from the swim bladder only the amount of oxygen that would be equivalent to the oxygen consumption of the fish over a period of three or four minutes.

The experiments reported in this paper show that the gases in the physostome swim bladder are fairly independent of the gases in the water and that the oxygen in the swim bladder is not readily mobilized during asphyxiation. The gases of the physoclist swim bladder, however, are in greater harmony with the external and probably the internal environments. Oxygen decreased during asphyxiation though its value to these fish for respiration is probably insignificant.

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